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CONTENTS

PAGE

GRIFFITHS, D. J. Light-induced Cell Division in *Chlorella vulgaris*, Beijerinck (Emerson Strain). With five Figures in the Text 85

DALE, J. E. Investigations into the Stomatal Physiology of Upland Cotton. 2. Calibration of the Infiltration Method against Leaf and Stomatal Resistances. With two Figures in the Text . . 94

WARREN WILSON, PAMELA M., and J. Cambium Formation in Wounded Solanaceous Stems. With six Figures in the Text 104

WELBANK, P. J. A Study of the Nitrogen and Water Factors in Competition with *Agropyron repens* (L.) Beauv. With five Figures in the Text 116

THOMAS, R. G. Correlations between Growth and Flowering in *Chenopodium amaranticolor*. I. Initiation of Leaf and Bud Primordia. With three Figures in the Text 138

CANNY, M. J. Measurements of the Velocity of Translocation 152

LOVELESS, A. R. A Nutritional Interpretation of Sclerophylly Based on Differences in the Chemical Composition of Sclerophyllous and Mesophytic Leaves. With three Figures in the Text . . 168

WELLINGTON, P. S., and DURHAM, V. M. Studies on the Germination of Cereals. 3. The Effect of the Covering Layers on the Uptake of Water by the Embryo of the Wheat Grain. With two Figures in the Text 185

DURHAM, V. M., and WELLINGTON, P. S. Studies on the Germination of Cereals. 4. The Oxygen Requirement for Germination of Wheat Grains during Maturation. 197

PLUNKETT, B. E. The Change of Tropism in *Polyporus brumalis* Stipes and the Effect of Directional Stimuli on Pileus Differentiation. With Plate and five Figures in the Text 206

CUNNELL, G. J. The Morphology of the Inflorescence in *Ranunculus bulbosus* L. With eight Figures in the Text 224

DORMER, K. J. The Crystals in the Ovaries of certain Compositae. With two Plates and twelve Figures in the Text 241

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Light-induced Cell Division in *Chlorella vulgaris*, Beijerinck (Emerson Strain)

BY

D. J. GRIFFITHS

Department of Botany,
University College of North Wales, Bangor

With five Figures in the Text

ABSTRACT

The inability of the Emerson strain of *Chlorella vulgaris* to grow and divide actively in a glucose medium in the dark has been confirmed. It has been shown that although glucose does not enhance the rate of cell-division when added to cultures growing under saturating photosynthetic conditions, it nevertheless markedly increases the growth-rate when supplied to cultures in which photosynthesis is limited by an inadequate CO_2 supply.

Transfer of actively growing cultures from light to darkness is followed by a limited period of active cell-division if glucose is added to the medium; this has been interpreted as indicating glucose utilization and the synthesis in light only, of some substance(s) essential for cell-division. Further evidence for this view has been obtained from studies of the effect of a light pretreatment on subsequent growth in the dark.

With cultures aerated with CO_2-free air, re-exposure to light after a period in the dark has been shown to bring about a resumption of active cell-division accompanied by a decrease in the percentage of 'giant' cells in the population. This also suggests the participation of some photo-reaction, other than photosynthesis, in the control of active cell-division in this strain of C. *vulgaris*.

INTRODUCTION

PRELIMINARY experiments in which the rates of cell multiplication of five different strains of *Chlorella vulgaris* were compared showed marked differences between the strains in their ability to grow in darkness. Using glucose (1 per cent.) as the source of carbon, it was noted that whilst four of the strains were able to utilize this sugar for growth and cell division in the dark, the fifth, the Emerson strain, showed little or no increase in population density after a period of 10 days in the dark. However, cultures of this strain, when subsequently returned to light were often able to resume active growth and cell division. These observations prompted a more detailed investigation of the response of this strain to added glucose.

EXPERIMENTAL

General experimental procedures

The organism, *Chlorella vulgaris*, Beijerinck (isolated by Emerson) was obtained from the Culture Collection of Algae and Protozoa, Cambridge, and

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was maintained on agar slants in diffuse daylight. The agar medium contained in g./l.: peptone (bacteriological), 1·0; KNO_3, 0·2; K_2HPO_4, 0·02; $MgSO_4.7H_2O$, 0·02; agar, 10·0.

Cells for initiation of experiments were washed from the slant and suspended in a sterile inorganic medium in the dark for 24 hours. The 'starved' cells were then settled out and, after discarding the supernatant medium, resuspended in sterile inorganic medium. One drop of this suspension (0·05 ml.) was transferred under aseptic conditions to each experimental tube to give an initial population density of 1 to 5 cells/mm.^{3}

The culture vessels were 50 ml. Quickfit tubes fitted with ground-glass attachments through which passed two delivery tubes plugged with cotton-wool. Each tube contained 20 ml. Craig and Trelease (1937) medium modified by using ferric ethylene diamine tetraacetic acid (5·4 mg. disodium Fe complex/l.) as iron source, by addition of molybdic acid (0·017 mg./l.) and by replacement of the phosphate by Sørensen's phosphate buffer of pH 5·20 (0·023 M $Na_2HPO_4.2H_2O$: KH_2PO_4 mixture) giving the equivalent concentration of phosphate in the medium. The experimental tubes containing medium were sterilized by autoclaving at 15 lb./in.^{2} for 10 minutes.

The cultures were incubated at 25° C. in a water bath and illuminated by 'daylight' fluorescent lamps giving an intensity of approximately 200 ft. candles at the surface of the culture. Each tube was aerated by attaching the delivery tube to a manifold connected to a compressed air supply. This continuous aeration (50 ml./min.) also ensured an even suspension of the algal cells. When aeration with CO_2-free air was required, the air was bubbled through three Drechsel bottles fitted with sintered-glass deliveries and containing respectively 10 per cent. NaOH, lime-water and water at a temperature of 25° C. 'Dark cultures' were grown in similar tubes enclosed in black-cloth covers many layers thick.

The progress of growth was followed by determination of the cells/mm.^{3} of medium using a Burker-ruled haemocytometer slide. A minimum of three tubes were removed for each estimation. In the growth curves, the cell numbers have been expressed on a $\log_{10}$ basis so that a straight line represents an exponential increase in population density. The rate of cell division (K) has been expressed as $\log_{10}$ daily increase in population density ($K = \log_{10} N_x - \log_{10} N_{x-1}$). Measurements of cell-size were made using a micrometer eye-piece which had been calibrated against a stage micrometer. Cell-size distributions within any one culture were obtained by measurement of all cells encountered in one or more of the areas of the haemocytometer slide.

Glucose utilization in the light

Inclusion of glucose (1 per cent.) in the medium of aerated cultures growing in the light did not bring about any increase in the rate of cell division (K inorganic medium = 0·74, K glucose medium = 0·69), whereas cultures aerated with a continuous stream of CO_2-free air were markedly affected by the presence of glucose (Fig. 1*a*). Cultures in an inorganic medium in the light, aerated with CO_2-free air, showed a continuously declining rate of cell division until a population density of about 90 cells/mm.^{3} had been achieved when the individual cells were characterized by a loss of green colour. Cell division could not be restored by reaerating the cultures with air containing CO_2. Cultures supplied with glucose and aerated with CO_2-free air, showed no such decline, the rate of cell division remaining almost constant throughout the 11 days of the experimental period giving a final population density of approximately 80,000 cells/mm.^{3} ($K = 0{\cdot}65$).

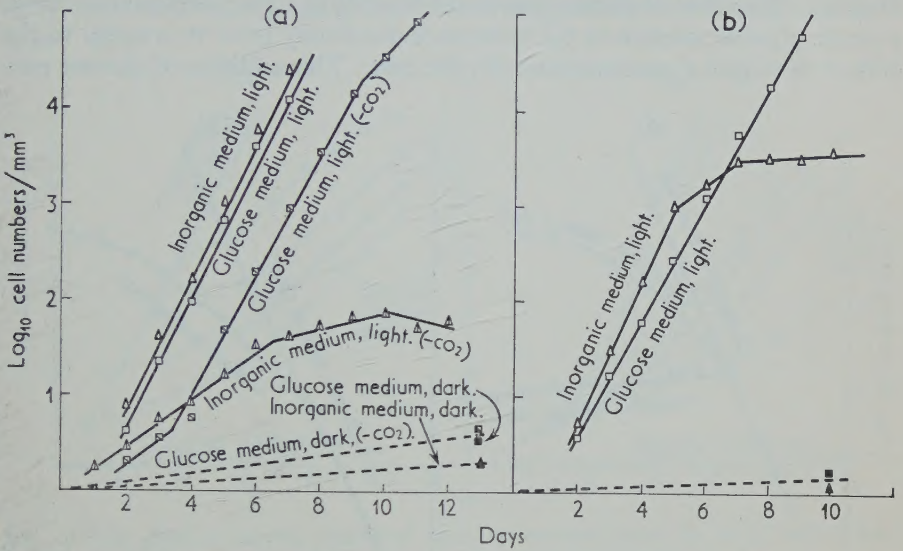

FIG. 1. The effect of glucose (1%) on the rate of cell division of *Chlorella vulgaris* (Emerson) under different conditions.

(*a*) Medium buffered at pH 5·20. (*b*) Medium buffered at pH 6·00. The broken line represents dark cultures in glucose or inorganic media.

Cultures supplied with glucose in the dark had a final population density of less than 10 cells/mm.^{3} after 13 days' growth with either method of aeration. The individual cells of these cultures, however, unlike those kept in an inorganic medium in the dark, appeared to have retained a healthy colour and were in fact capable of renewed active cell division when returned to the light.

Emerson and Green (1938) reported that at a pH 6·00 less than 50 per cent. of the total CO_2 of their culture medium was in the form of free CO_2. The effect of such a high pH on the rate of cell division of aerated cultures in the present series of experiments is shown in Fig. 1*b*. For this experiment a phosphate buffer of pH 6·00 replaced the usual phosphate mixture. When cultures were grown in an inorganic medium in the light, the initial rate of cell division ($K = 0{\cdot}77$) fell off rapidly at a population density of about 1,000 cells/mm.^{3} when, presumably, carbon dioxide had become a limiting factor. Similar cultures supplied with glucose, however, maintained a steady rate of cell division for about 9 days ($K = 0{\cdot}61$) until a population density of about

70,000 cells/mm.^{3} had been achieved. No significant cell multiplication was observed in cultures supplied with glucose in the dark.

Glucose utilization in the dark

Cultures which had been growing in an inorganic medium in light for 4 days, when transferred to the dark, showed an immediate cessation of cell division. The effect of glucose was tested by adding, under aseptic conditions, a sterile glucose solution to the cultures immediately prior to transfer to the dark so as to give a concentration of 1 per cent. This addition of glucose permitted a continuation of cell division ($K = 0{\cdot}40$) in the dark although at a rate appreciably below that achieved under photosynthetic conditions ($K = 0{\cdot}75$) and which, after the first two days in the dark, showed a gradual decline (Fig. 2*a*). A similar result was obtained when the 4 days' light pretreatment was given to cultures in a 1 per cent. glucose medium (Fig. 2*b*). In both instances, the fall-off in the rate of cell division was accompanied by a marked increase in the percentage of large 'giant' cells in the population (i.e. cells having diameters greater than 30μ as compared with diameters of between 5 and 10 μ in light-grown cells).

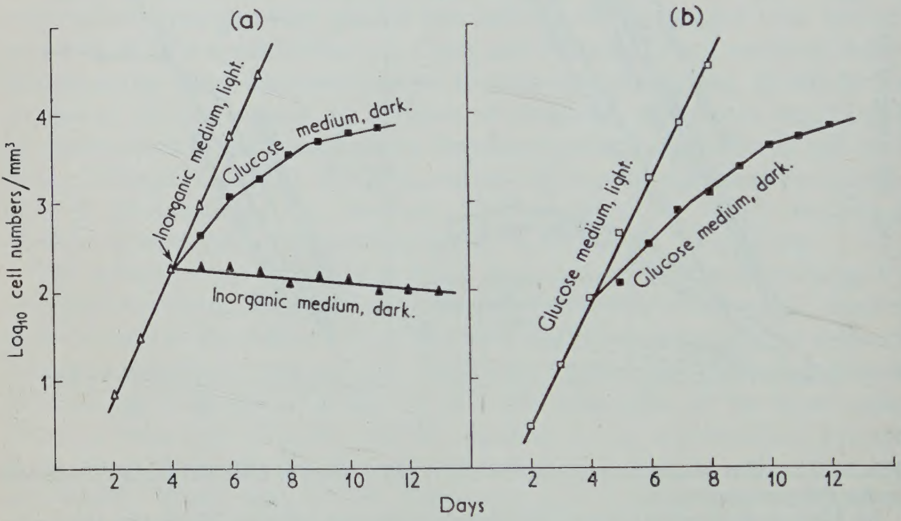

FIG. 2. The rate of cell division in darkness of *Chlorella vulgaris* (Emerson) in a glucose (1%) medium following transfer from light.

(*a*) 4 days' light pretreatment in an inorganic medium.

(*b*) 4 days' light pretreatment in a glucose (1%) medium.

The effect of a 'pretreatment' with light on subsequent growth in a glucose medium in the dark

Cultures growing in an inorganic medium were exposed to light for varying periods and their subsequent behaviour in darkness after addition of glucose observed (Fig. 3*a*). Light pretreatments of 1, 2, 3, and 4 days were equally effective in inducing cell division in the dark although the average rate of cell division achieved ($K = 0{\cdot}35$) was appreciably lower than the rate in the corresponding photosynthetic cultures ($K = 0{\cdot}62$). The duration of the light pretreatment had a marked effect on the extent of the lag period prior to the establishment of a steady rate of cell division in the dark. There was some evidence of a fall-off in the rate of cell division in cultures exposed to the longer pretreatments (3 and 4 days) with a slight increase in the percentage of 'giant' cells.

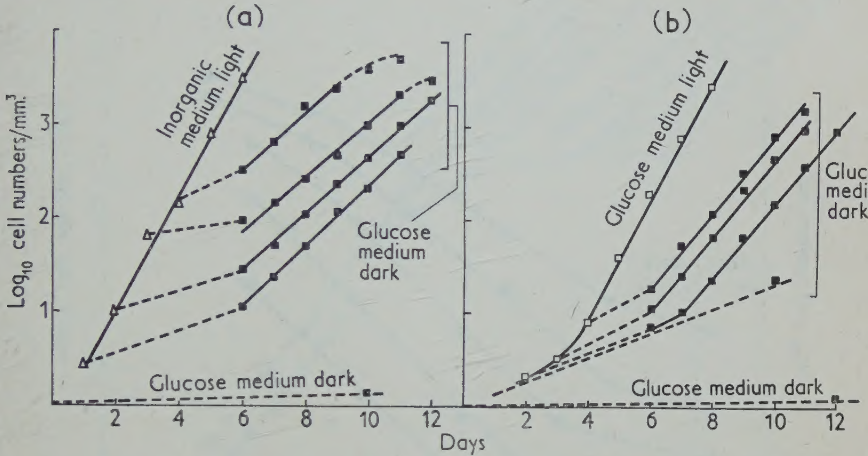

FIG. 3. The effect of varying periods of light pretreatment upon the rate of cell division in darkness of *Chlorella vulgaris* (Emerson) in a glucose (1%) medium.

(*a*) Light pretreatment in an inorganic medium.

(*b*) Light pretreatment in a glucose (1%) medium.

Light pretreatment in a 1 per cent. glucose medium was also effective in promoting cell division in the dark (Fig. 3*b*). Again, the average rate of cell division in the dark ($K = 0{\cdot}40$) following light pretreatments of 2, 3, and 4 days was appreciably lower than the rate in the light ($K = 0{\cdot}68$). The results obtained with a light pretreatment of 1 day, together with the variation in the extent of the lag period following the different pretreatments noted previously rendered it desirable to test the effect of the population density at the time of transfer upon subsequent growth in the dark (Fig. 4). The cultures were initiated with inocula giving initial population densities of 44, 12, 6, 2, and 1 cells./mm.^{3} and subjected to a light pretreatment of 6 hours in a 1 per cent. glucose medium before transfer to the dark. At the lower initial population densities (1 and 2 cells/mm.^{3}) there was a relatively long lag before active cell division was established but the rate of cell division of these cultures subsequent to the lag period was nevertheless of the same order as previously noted for growth on glucose in the dark ($K = 0{\cdot}37$ and $0{\cdot}36$ respectively). Cultures with the higher initial population densities (12 and 44 cells/mm.^{3}), although having a much shorter lag phase, showed a distinct fall-off in the rate of growth accompanied by an increase in the percentage of 'giant' cells towards the end of the experimental period.

The effect of exposure to light after a period in the dark

Cultures growing in a 1 per cent. glucose medium in the dark following a 1-day light pretreatment maintained a steady rate of cell division for only about 12–14 days. At this time, when the population densities were between 1,000 and 3,000 cells/mm.^{3}, there was a marked decline in the rate of cell division accompanied by an increase in the percentage of 'giant' cells in the cultures. The effect of re-exposing such cultures to light is shown in Fig. 5.

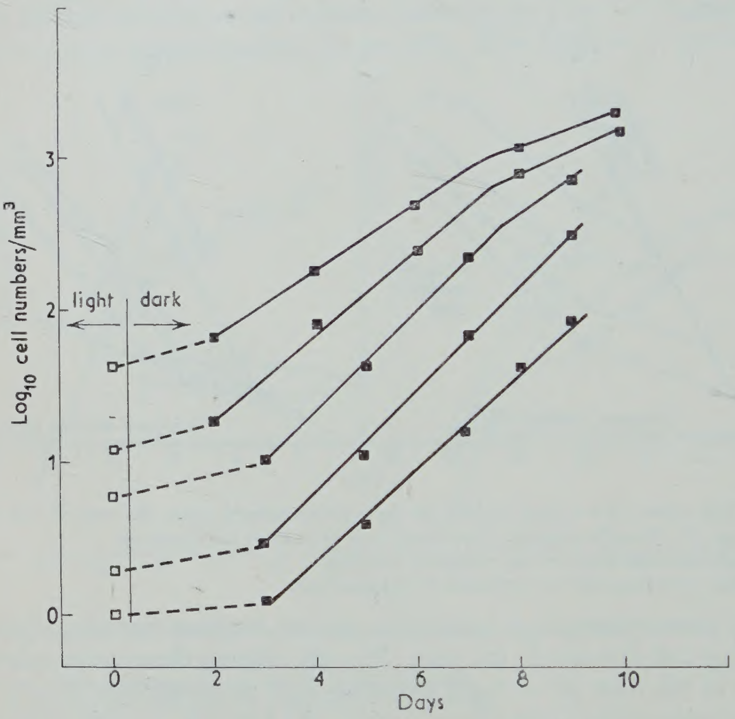

FIG. 4. The effect of the initial population density upon the rate of cell division in darkness of *Chlorella vulgaris* (Emerson) in a glucose (1%) medium following a 6-hr. light pretreatment.

Re-exposing the cultures to light after 12 days in the dark prevented the onset of the phase of declining rate of cell division. This effect was noticed irrespective of whether the re-exposed cultures were aerated or supplied with CO_2-free air. After 16 days' growth in the dark, when cell division had almost completely ceased, re-exposure of the cultures to light brought about an immediate recovery in the rate of cell division with both forms of aeration.

Estimation of cell size after 16 days' growth in the dark showed that 'giant' cells of diameters greater than 30 μ formed, on average, approximately 20 per cent. of the population (population density approximately 2,500 cells/mm.^{3}). Re-exposure of cultures in this stage to light brought about a marked decrease in the percentage of 'giant' cells in the population. Cultures which had been returned to light for 4 days (population density approximately 20,000 cells/

mm.^{3}) had less than 1 per cent. of their population in the form of giant cells. This would seem to indicate that re-exposure to light not only prevents the appearance of more 'giant' cells, but also brings about a decrease in the number of 'giant' cells in the population.

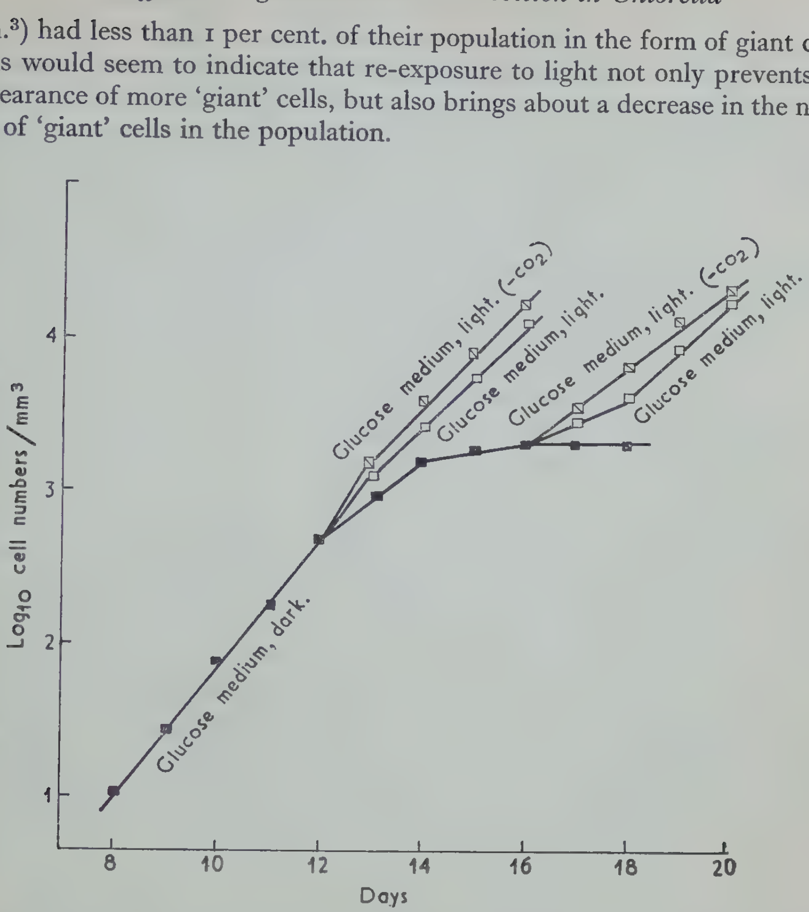

FIG. 5. The effect of exposure to light upon the rate of cell division of *Chlorella vulgaris* (Emerson) grown in darkness in a glucose (1 %) medium following a 1-day light pretreatment.

DISCUSSION

The results presented in this paper show the necessity of a light reaction for active cell-division of the Emerson strain of *Chlorella vulgaris* under conditions where externally fed glucose is used as the major source of energy and carbon. In this respect, this strain would appear to differ from the Brannon strain which can undergo active cell-division in the dark in a glucose medium (Griffiths, Thresher, and Street, 1960).

The inability, here reported, of the Emerson strain to multiply actively in a glucose medium in the dark confirms the report of Finkle, Appleman, and Fleischer (1950) and Killam and Myers (1956).

It has been shown that although glucose is without effect upon the rate of cell-division when added to cultures growing under saturating photosynthetic conditions, it nevertheless has a marked effect when added to cultures in which photosynthesis is limited by an inadequate CO_2 supply. Clearly, therefore, there is no difficulty in glucose utilization in the light, and the recovery shown by dark cultures when returned to the light would seem to suggest that glucose assimilation is also possible, at least to some extent, in the dark. Pearsall and Bengry (1940) reported a marked stimulation of the growth-rate of *C. vulgaris* (Hopkins and Wann strain) in a glucose medium by very low light intensities, but with only a small response to increasing light intensities. This they interpreted by supposing that even weak light produces some alteration in protoplasmic state which permits all metabolic reactions to proceed at a much higher rate. Killam and Myers (1956), working with the Emerson strain of *C. vulgaris*, found that an added light dosage, so small that it would support no growth in the absence of glucose, caused a large increase in the rate of cell proliferation and interpreted the light effect as a stimulation of the synthesis of some key materials required for cell division. The results of the present study would seem to confirm these views of a special response to light in this alga.

A limited period of active cell multiplication in a glucose medium in the dark followed transfer of actively growing light cultures and was observed irrespective of whether the light cultures were in an inorganic medium or in a glucose medium. This might be interpreted as being due either to the increased possibility of production in an actively growing culture of suitable variants for growth and multiplication in the dark, or to a synthesis during the light period of some key substances required for cell-division. The fairly early fall-off in the rate of cell-division during the subsequent dark period and the accompanying appearance of the 'giant' cells, clearly point to the latter as the more likely explanation. Further confirmation of this view is given by the results of pretreatment experiments where exposure of cultures of very low population densities (1 cell/mm.^{3}) to relatively short light pretreatments (6 hours) is sufficient to induce active cell-division for a limited period in a glucose medium in the dark. Observation of the effect of population density at the time of transfer would seem to suggest that the greater the density of the population exposed to the light pretreatment, the sooner does the effect of the pretreatment disappear.

The recovery shown by cultures upon re-exposure to light after a period in the dark and the gradual decrease in the percentage of 'giant' cells following re-exposure agree with the hypothesis put forward and point toward the participation of some photo-process in the control of active cell division. The independence of the light-reaction upon CO_2 supply clearly differentiates it from photosynthesis unless respiratory CO_2 is adequate in amount for the synthesis of the active compound. Tamiya, Iwamura, Shibata, Hase, and Nihei (1953) have suggested that the life cycle of *Chlorella* can be divided into two phases: an assimilation phase during which growth in size of the cell occurs, and a division phase during which the enlarged cell divides into a number of autospores. Myers (1957) has further shown that for cells growing photosynthetically, only the assimilation phase is light-dependent. The results of the present investigation would seem to suggest that in the Emerson strain of *C. vulgaris*, it is the division phase which is light-dependent, carbon assimilation from glucose being apparently possible in complete darkness. Clearly a further study of the effect of pretreatment with light is required, particularly a determination of the action spectrum of the light reaction, which would constitute the most critical test of its distinction from photosynthesis.

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Investigations into the Stomatal Physiology of Upland Cotton

2. Calibration of the Infiltration Method against Leaf and Stomatal Resistances

BY

J. E. DALE

Empire Cotton Growing Corporation,
Cotton Research Station, Namulonge, Uganda

With two Figures in the Text

ABSTRACT

Data are presented to show that reductions in leaf water content of up to 12 per cent. are associated with increases of mesophyll resistance to viscous flow of air of from 50 to 130 per cent. This effect may explain the variation observed in the relationship between Infiltration Score (I), and the logarithm of leaf resistance, measured porometrically, for leaves with different water contents.

Highly significant linear correlations were obtained by plotting the logarithm of the resistance of 10,000 stomata against I for leaves from plants grown in the field ($r = -0{\cdot}92$) or in the greenhouse ($r = -0{\cdot}94$). For the two sets of material the slopes of the lines were similar, but a difference in levels was highly significant. This is considered in relation to leaf and stomatal structure. The use of the infiltration method for deriving estimates of stomatal resistance is discussed.

INTRODUCTION

IN a previous paper (Dale, 1961) data were presented to show the effects of hour of day, solar radiation, temperature, and relative turgidity on stomatal aperture, estimated using an infiltration technique, for cotton grown under field conditions.

Although a number of attempts have been made to calibrate stomatal dimensions with porometer results (see Heath, 1941, pp. 456–7) few, if any, records exist of attempts to calibrate infiltration data with leaf or stomatal resistance. This is probably because the infiltration method has been considered to be inaccurate and less useful quantitatively than the epidermal strip method (Lloyd, 1908). Unfortunately the strip technique cannot be used for cotton, since the epidermes adhere closely to the underlying tissues.

In a recent review Milthorpe (1959) suggested that valuable information on stomatal behaviour can be obtained by calibrating the infiltration method against a resistance porometer. Such a calibration has been attempted in the work described here.

[Annals of Botany, N.S. Vol. 25, No. 98, 1961.]

METHODS

A Wheatstone Bridge porometer (Heath and Russell, 1951) was used in most of the experiments, having been constructed in the Botany Department, Makerere College, with the kind assistance of Mr. T. R. Milburn. The capillary resistances used in the instrument, together with three needle valves (Edwards and Co., Type L.B.I.), were calibrated in terms of Gregory and Pearse units (Heath, 1941) using a flow-meter. Two of the needle valves gave calibration curves similar to that shown by Heath and Russell (1951), but the third valve gave only a seventyfold variation in resistance and was not used. One valve was found to stick after prolonged use and after lubrication its characteristics were slightly altered. From time to time therefore, valve calibrations were checked using known resistances. The air supply to the instrument came from a Dymax air compressor, with an escape valve, controlled to give a pressure equivalent to about 6 cm. of water.

In most experiments a simple leaf chamber of perspex with a detachable lid, and fitted with a porometer cup on a screw plunger, was used. The cup was sealed to the leaf by an annular washer (width 4 mm.) of 35 per cent. gelatine/glycerine, lightly greased with petroleum jelly and enclosing an area of 122·7 $mm.^2$

Preliminary experiments were performed in a darkened room (temperature 22–24° C.) using as a light source a 1,000 watt tungsten filament lamp, held behind a water screen, 50 cm. from the leaf being investigated. Subsequent experiments were performed in a laboratory lit by diffuse daylight and having a temperature of 24–27° C.

The technique of infiltration with *iso*-butanol and ethylene glycol and the relative turgidity method used have been discussed in the earlier paper. Other techniques are described in the appropriate part of the text.

MATERIAL

Plants used in this work came from the Modal Bulk series of the BP52 variety (Hutchinson and Manning, 1951). Mature leaves borne on the main stem of plants 10–16 weeks old were used. The leaves are trilobed, and in all studies the area investigated was in the middle lobe on either side of (but not including) the main vein.

RESULTS

1. *Preliminary studies and experiments on mesophyll resistance*

Comparison of results from infiltration and porometric studies rests on the assumption that attaching or detaching a porometer cup to a leaf will not alter the measured resistance, of leaf (R_L), or stomata (R_S), during, or immediately after the reading, and that handling will not affect the Infiltration Score (I) disproportionately to the resistance. This assumption was tested in a series of experiments in which leaves were infiltrated before and after attachment of the cup, and for leaves to which the cup was repeatedly attached or detached.

Handling effects were not detectable using the infiltration method, and were found to cause a slight, ephemeral closure, lasting about 4–5 minutes, when estimated porometrically. Yemm and Willis (1954), using *Chrysanthemum maximum*, found a marked closure following cup attachment. In their experiments, in contrast to the present ones, the cup remained attached for long periods and this may have been associated with the closure (Williams, 1949).

A further complication in comparative experiments is that because of the injurious effects of the infiltrating agents, any leaf can only be used for a single comparison. In order to compare a range of values of R_L or R_S and I it is therefore necessary to assume that different leaves will show the same relationship between the two factors. It will be shown below that this assumption is only valid when leaves of similar status are used.

In preliminary comparative experiments, leaf resistance to viscous flow of air (R_L) was compared with I for intact leaves on plants grown under greenhouse conditions. The procedure followed was to place a mature leaf in the chamber, attach the porometer cup to the lower surface, and determine R_L. This procedure took 60–90 seconds. The cup was then detached and the leaf infiltrated at one point only on the lower surface to obtain an estimate of I. Results are given in Fig. 1 for two sets of leaves with relative turgidity greater than 90 per cent., or less than 85 per cent. It will be noted that for all values of I, except $I = 0$, leaf resistance for leaves with the higher turgidity is significantly lower than that for the less-turgid leaves. The range of values of R_L for given values of I is considerable for highly turgid leaves, suggesting that variation in the relationship between leaves of approximately similar water status may be considerable. This is perhaps due to differences in the range of stomatal apertures associated with the same maximum aperture, which mainly controls infiltration.

The differences in R_L for the two classes of leaves could be due either to variation in resistances to flow within the leaf, normal and parallel to the blade (mesophyll resistances) or to variation in the stomatal component of leaf resistance, due to differences in stomatal frequency associated with area changes. The effect of water strain on mesophyll resistance was investigated using a method due to Meidner (1955), in which air is drawn through a narrow ring of leaf tissue mounted between washers. The rate of passage of air through the tissue is measured by the time taken for a mercury slug to fall a given distance down a capillary tube; this is approximately proportional to the resistance applied, and thus gives a measure of the lateral mesophyll resistance of the annulus.

In the present experiments the tissue ring was placed on top of a gelatine washer and below a lightly greased cover slip on which was placed a 20 g. weight to ensure an adequate seal in the system. The pressure applied on the ring was about 20 g./cm.^{2}, whereas with the clamping device used by Meidner, pressures of the order of 200 g./cm.^{2} were used. The rings were cut from between the main veins of fully turgid leaves, and had external and internal radii of 9·0 and 7·0 mm. respectively. The 'falling time' for a ring of fully turgid tissue of known weight was determined and the ring allowed to lose water in position on the apparatus. After a variable amount of water had been lost the 'falling time' was again determined. Following this, the ring was weighed and the amount of water lost was calculated. Data in Table 1 show the changes in 'falling time' associated with changes in water content for 5 different tissue rings. In all cases a loss of water from the ring was related to an increase in the mesophyll resistance estimate.

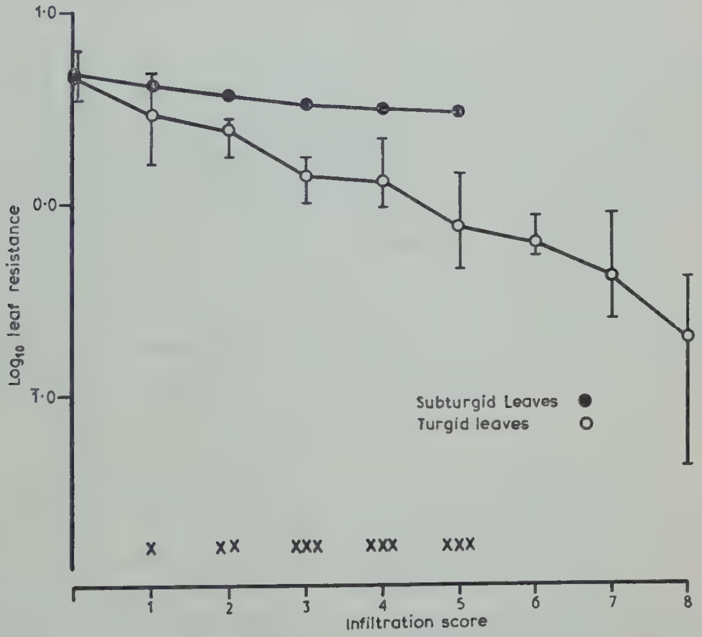

FIG. 1. The relationship between $\log_{10} R_L$ and I for leaves with different turgidities. For the 'turgid' leaves, which had values of relative turgidity greater than 90% vertical lines indicate the range of values obtained. Crosses below indicate differences significant at the 5% (×), 1% (××) or 0·1% (×××) level.

Meidner (1955) suggested that the increases in mesophyll resistances might be due to shrinkage of the internal air spaces of the leaf. Attempts were made to discover whether there was a decrease in the intercellular space volume for cotton leaf tissue suffering water strain. Both leaves, and leaf disks, of known water content were injected under reduced pressure with petroleum ether (80–100° C. B.Pt.) and the volume of the injecting fluid determined by weighing. Results indicated that the intercellular space volume is reduced from about 17–18 per cent. to about 13–15 per cent. of the total leaf volume for a reduction in leaf water content of 15 per cent. Both whole leaves and disks of leaf tissue showed quantitatively similar reductions in intercellular space volume with water stress.

TABLE I

The Relation between Water Loss and Increase in 'falling time' for Rings of Leaf Tissue. 5 Experiments

Run	Loss of water (%)	Increase in falling time (%)
1	3·1	57
2	6·8	90
3	12·2	51
4	12·4	86
5	12·0	129

2. *The relationship between stomatal resistance and I*

Leaf resistance by itself is clearly an unsatisfactory factor to correlate with I since it is the resultant of a number of resistances within the leaf. I should more correctly be related to stomatal resistance alone. The most satisfactory way of estimating this is by a double-cup method (Heath, 1941). More recently, however, Heath and Milthorpe (1950) by-passed the problem of allowing for lateral flow in the leaf by using leaves with a ratio for upper to lower stomatal numbers which was greater than unity. Lateral flow effects then became negligible (Heath and Russell, 1954, p. 289) and by neglecting also mesophyll resistances normal to the leaf surfaces it was possible to calculate $R_{10,000}\,S$, the resistance to flow offered by 10,000 stomata, from the equation

$$\log_{10} R_{10,000}\ \text{Stomata} = \log_{10} R_L + \log_{10}\left(\frac{n_u n_1}{n_u+n_1} \times \frac{10{,}000}{n_u^1+n_1^1}\right),$$

where R_L is the observed leaf resistance, n_u and n_1 are the observed stomatal numbers on the upper and lower surface, having a ratio α, and n_u^1 and n_1^1 are the corresponding numbers to give a total of 10,000 and a ratio of α. At the suggestion of Professor Heath attempts were made to relate $\log_{10} R_{10,000}\,S$ with I. The procedure was as follows. The leaf under study was placed in the perspex chamber, the porometer cup attached to the upper surface of the leaf (i.e. the surface with fewest stomata, making $\alpha > 1{\cdot}0$), and R_L determined. Immediately afterwards I was determined by applying solutions at four points adjacent to the area enclosed by the cup. The mean of the four values was used in subsequent comparisons. The tissue within the cup was then removed and its area estimated by projection on to graph paper using a photographic enlarger. This precaution was necessary since distortion of the standard-size gelatine washer occasionally occurred. Stomatal frequencies for each surface were determined by counting and from these data $\log_{10} R_{10,000}\,S$ was calculated. Most of the leaves were excised and allowed to become fully turgid before investigation, but some leaves from greenhouse plants were allowed to lose turgor to correspond with the subturgid leaves used in the earlier experiments. Results are shown in Fig. 2.

For leaves from greenhouse-grown plants, a straight line has been fitted relating the two variables ($r = -0{\cdot}94$, $b = -0{\cdot}377$), and in contrast to the earlier results, water status does not influence this relationship. A straight line ($r = -0{\cdot}92$, $b = -0{\cdot}416$) has also been fitted to the data from plants grown in the field, although here for given values of I, $\log_{10} R_{10,000} S$ is lower

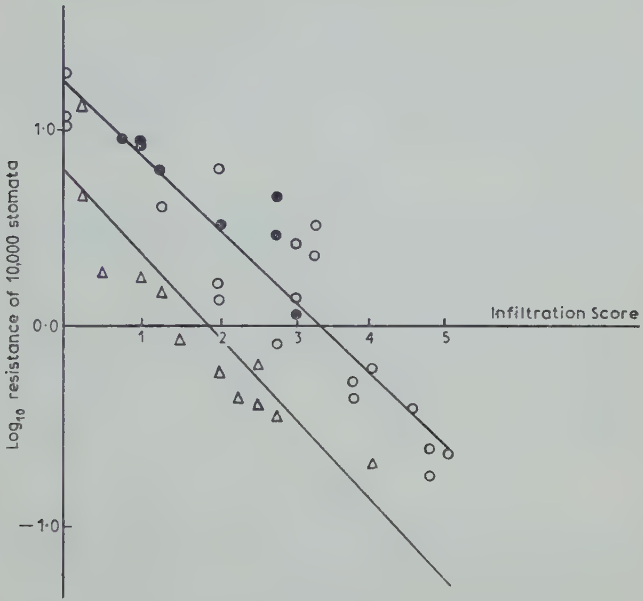

FIG. 2. The relationship between $\log_{10} R_{10,000} S$ and I for leaves of plants grown in the field (triangles) and in the greenhouse (circles). The solid circles refer to 'sub-turgid' leaves.

than that for greenhouse plants. This difference in levels was tested and found to be highly significant. The results of the analysis of variance are given below:

Source	D.F.	S.S.	M.S.	F	P
General Regression	1	8·72	8·72	189·56	< 0·001
Levels . . .	1	2·84	2·84	61·74	< 0·001
Slopes . . .	1	0·06	0·06	1·30	ns
Error . . .	36	1·67	0·046		

A fundamental assumption in the derivation of $R_{10,000} S$ is that resistances within the leaf are slight, and that stomatal resistances are the only significant factors contributing to the estimate. If this is true, differences between the two sets of leaves should be related to stomatal factors alone. These were therefore investigated.

(*a*) *Stomatal dimensions.* No evidence was found to suggest that pore width or length differed between leaves from the two sources. Values were similar to those quoted in the earlier paper. However, from microtome sections it was found that differences in pore depth occurred. The values recorded were 15·2 μ as a means for 18 estimates on greenhouse plants and 12·8 μ for 14 estimates on field plants. The difference was just significant at the 5 per cent. level of probability ($t = 2{\cdot}05$ for $n = 32$).

(*b*) *Stomatal frequency and values of* α. No significant differences were found when comparing values for fully turgid leaves from both sources (Table 2). Values of stomatal frequency per mm.^{2} are approximately 50 per cent. higher than those reported in the earlier paper, and this would seem to reinforce the conclusion then expressed that stomatal frequency varies between limits depending on leaf position on the plant, season, and locality.

TABLE 2

Values for Stomatal Frequency per Cup (area = 122·7 mm.^{2}) and of α for Leaves from Greenhouse and Field-grown Plants

	Frequency		
	Upper surface	Lower surface	α
Greenhouse	9,500	20,100	$2{\cdot}16 \pm 0{\cdot}23$
Field .	10,200	20,900	$2{\cdot}06 \pm 0{\cdot}20$
t ($n = 40$) .	0·92 ns	1·11 ns	1·28 ns

TABLE 3

Comparison of Tissue Thickness (in μ) for leaves from Greenhouse and Field-grown Plants, and the Significance of Differences

	Greenhouse	Field	t	P
Total thickness .	168	194	12·86	0·001
Upper epidermis	18·8	20·6	2·89	0·01
Lower epidermis	18·6	18·9	0·35	n.s.
Palisade . .	74·3	83·4	2·38	0·05
Mesophyll .	56·5	71·4	5·47	0·001

(*c*) *Differential response to infiltration between the two sets of material.* The possibility that given values of I were associated with different stomatal apertures for the two sets of material was investigated using direct observation of the pore by the method described in the earlier paper. No significant differences were observed.

To check that certain other aspects of leaf structure were not associated with the differences between the two classes of material two further factors were examined.

(*d*) *Intercellular space volume.* This was determined using the injection technique already described. Values, in arbitrary units, for the volume of intercellular space for disks of standard area cut from fully turgid leaves were:

Greenhouse plants	$1{\cdot}28 \pm 0{\cdot}26$
Field plants	$0{\cdot}56 \pm 0{\cdot}17$

The difference is highly significant ($t = 7{\cdot}22$, for $n = 36$).

(*e*) *Leaf thickness.* The relative thickness of the tissues within the leaf was determined using microtome sections cut from 10 leaves from each source.

Data given in Table 3 show that leaves from field plants are significantly thicker than those from greenhouse plants, and that this difference is associated with significantly greater thickness of all tissues except the lower epidermis.

DISCUSSION

In early attempts to compare porometric results with those from other methods for studying stomata (e.g. Paetz, 1930; Ashby, 1931) leaf resistance was used as the porometric estimate. This was unsatisfactory since such values were influenced by variations in both mesophyll resistance and stomatal frequency.

From experiments using tissue rings it is shown that water losses of up to 12 per cent. are associated with increases in the mesophyll resistance estimate of from 50–130 per cent. These values are of the same order as those quoted by Meidner (1955) for *Psychotria* and *Scolopia.* Data from the intercellular space volume determinations support the conclusion that mesophyll resistance increases rapidly as leaf water content declines. Not only is the intercellular space volume reduced as a percentage of the total volume, but the absolute volume is reduced by 25–35 per cent. for a 15 per cent. loss of water (cf. data of Kennedy and Booth, 1958). Such large decreases might be expected to have marked effects on mesophyll resistance.

In the experiments comparing R_L with I, the value of α was always less than 1·0 since the porometer cup was attached to the leaf surface having greatest stomatal frequency. It seems reasonable to conclude that differences in mesophyll resistance would markedly affect the estimate of R_L and that such variation can largely account for the differences observed between the two classes of material (Fig. 1). Unfortunately, data for stomatal frequency in turgid and subturgid leaves were not obtained so that it is impossible to assess the amount of variation in R_L attributable to this cause.

For the determinations of stomatal resistance, where α always exceeded 1·0 by a relatively large amount, differences in the correlation due to variations in relative turgidity were not found. In so far as one can argue from cotton to wheat leaves this is indirect evidence that the assumption made by Heath and Milthorpe (1950), that intercellular space resistance is negligible normal to the leaf blade, is correct, since by computing the resistance for 10,000 stomata, differences due to variation in stomatal frequency are ruled out.

It is not easy to explain the significant differences in the relationship between $\log_{10} R_{10,000} S$ and I for leaves from greenhouse and field plants. Since differences in stomatal frequency and values of α are slight, the differences in the relationship should be attributable to variation in dimensions of either the stomata or the leaf. The observed differences in leaf thickness and intercellular space volume are such as to make resistance to flow normal to the leaf greater for field material; in fact $R_{10,000} S$ is less for these leaves. This also supports Heath and Milthorpe's assumption that resistances normal to the leaf are unimportant.

The unexplained difference in level between the two regression lines is curious rather than important and it is unlikely that the infiltration method would be used in attempts to compare *absolute* values in two such different environments as field and greenhouse. The similarity of slopes is encouraging and suggests that the method may be used for comparative tests within widely different environments.

As pointed out by Heath and Russell (1954) log resistance of the stomata to viscous flow of air is in no way a fundamental measure, as would be stomatal resistance to diffusion if interest were centred on stomatal control of transpiration or assimilation, or turgor differences between guard cell and subsidiary cell if stomatal mechanism was the main interest. In most field studies, however, the object is to relate stomatal aperture to external conditions and often to use it as an indication of water strain or irrigation need. For such purposes any convenient method that gives a consistent measure of aperture little affected by other changes in the leaf is suitable. The results presented in this paper suggest that the infiltration method, which is undoubtedly the most convenient, is sufficiently accurate for many such purposes.

ACKNOWLEDGEMENTS

I am very grateful to Professor O. V. S. Heath, F.R.S., for many helpful criticisms on the text, and also during the course of this work. Thanks are also due to my colleagues at Namulonge for discussion of the text.

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Cambium Formation in Wounded Solanaceous Stems

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With six Figures in the Text

ABSTRACT

When stems of certain Solanaceous species are wounded the internal phloem strands that lie near to the damaged surface become enlarged through cell division, and some of them develop a vascular cambium. This 'internal cambium' forms phloem inwards and xylem outwards; it is thus in inverted orientation. Such a structure does not occur in the normal stem of any Solanaceous plant. Further, during the regeneration of a cambial cylinder after wounding, the regenerating cambium may turn inward to meet wounded internal phloem strands. Where this happens, subsequent secondary thickening leads to the formation of thin tubes of cambium running radially through the xylem and forming a limited amount of enclosed phloem which connects the internal and external phloem systems of the stem.

The positions in which these cambia arise are explicable by the 'gradient induction' hypothesis of cambial regeneration, but not by the 'free surface' and 'cambial ring' theories.

NORMAL STATUS OF INTERNAL PHLOEM

INTERNAL phloem occurs in the stems of all Solanaceous plants; it lies close to the protoxylem, but generally separated from it by a narrow layer of ground parenchyma. In the species with which we are concerned here (*Atropa belladonna*, *Datura stramonium*, and *Lycopersicum esculentum*) it consists of numerous thin strands running longitudinally and occasionally anastomosing; strands in transverse section are shown in Fig. 1. This internal phloem is generally regarded as primary in origin; it arises at the inner margin of the procambial ring by the irregular longitudinal division of certain ground-meristem cells to form procambial strands in which the cells differentiate centrifugally into phloem elements (Baranetzky, 1900; Esau, 1938). However, in a small number of Solanaceous species these phloem strands may subsequently become augmented through the cells on their outer edges dividing in a rather orderly manner, with tangential walls predominating, and differentiating (again centrifugally) into phloem elements. In such cases the meristematic cells resemble cambium (Vesque, 1875; Esau, 1938); but since xylem is never produced by this meristem it has been termed a 'false cambium' or 'unilateral cambium' by Vesque and Baranetzky. In most Solanaceous species no unilateral cambium occurs; sometimes there is rather irregular augmentation of the internal phloem at its outer edge; often there is no evidence even of this (Artschwager, 1918; Woodcock, 1935). In our material

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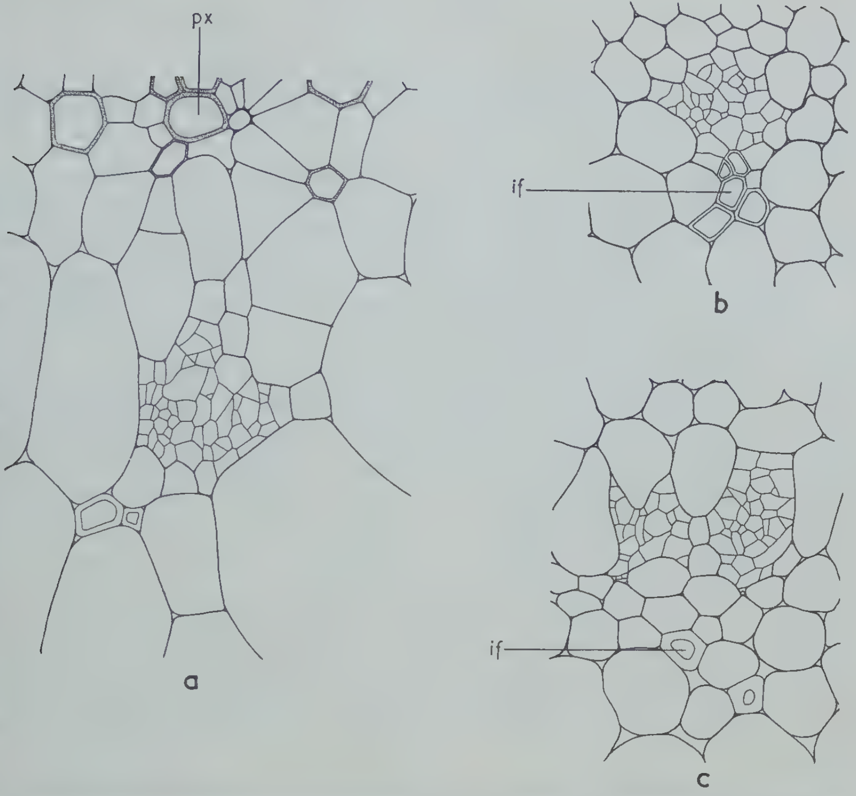

Fig. 1. Normal internal phloem strands in T.S. (*a*) *Datura*; (*b*) *Atropa*; (*c*) *Lycopersicum*; *if*, internal fibres; *px*, protoxylem.

some internal phloem strands show a preponderance of tangential walls at their outer edge, but there is never sufficient orderliness to justify the use of the term 'unilateral cambium'.

FORMATION OF INTERNAL CAMBIUM AND XYLEM AFTER WOUNDING

Our observations have been made on (i) parts of internodes that have been wounded when young by a longitudinal cut removing about one-third of the thickness of the stem over a distance of 2 or 3 centimetres, and (ii) approach-graft unions made by tying together either two stems wounded in this way, or a wounded stem and a wounded petiole. The operations were performed in early summer on plants growing rapidly in a greenhouse; the plants were sampled some 4 to 20 weeks later. Sections of each wound or graft union were cut with a sliding microtome at about 70 μ without embedding, and stained with safranin and light green. The anatomical changes described below were essentially similar for both wounds and grafts and for all three species examined; accordingly no distinction on these bases is made in the following account.

Except for Figs. 4*a* and 6*a*, all the illustrations have been made with a camera lucida to the following scales: ×10 for diagrams and ×200 for drawings.

Wounding of the stem causes cells near the cut surface to proliferate freely. Internal phloem cells take part in this activity, usually over a greater distance from the cut surface than is the case for ground tissue; those phloem strands that lie nearest to the cut surface tend to be most affected, those that are more distant being progressively more normal in structure. In strands that are relatively little affected there is found merely a slight increase in the thickness of the strand as a result of increased cell division. Other strands show a more marked increase in both tangential and radial dimensions and may have on their outer margin a more or less well-defined unilateral cambium giving rise to files of cells that differentiate into phloem elements (Fig. 2*a*). Close to the wounded surface some phloem strands may develop what is properly termed a true cambium, since it gives rise not only to phloem but also (from its other face) to xylem, the cells of these tissues showing more or less orderly radial seriation throughout. We term this an internal cambium, and the tissues derived from it internal xylem and internal phloem.

Generally transverse sections show only a few xylem elements formed in association with a single internal phloem strand (Fig. 2*b*), but occasionally, near the cut surface, a considerable thickness of internal xylem occurs (Figs. 3*a*, 3*b*).

Apart from occasional aberrant cases, which are sometimes the result of active cell proliferation, the internal cambium regularly produces phloem on its inner side and xylem outwards. Thus in comparison with normal vascular cambia it is radially inverted in orientation.

Since internal xylem is formed in only those internal bundles that lie close to the wound surface, and since even here its occurrence is spasmodic, it is generally found only in small amounts in any one graft union or wound. However, it occurs fairly consistently; we find it in 44 out of 60 graft unions and wounds examined.

This internal xylem is quite distinct from the 'xylem strands' that often arise around wounds and in graft unions and are composed of tracheids or vessels formed by lignification of callus or ground tissue (e.g. Crafts, 1934; Jacobs, 1952). Such xylem strands occur occasionally in our material, but differ from internal xylem in that the elements are roughly isodiametric, usually follow a rather oblique course, and are not constantly associated with internal phloem.

The internal xylem in our plants can be seen in longitudinal sections to consist of lignified elements joined end to end in a series that is fairly well organized though less precisely arranged than normal xylem tissue. Although these series are often only one or a few cells thick they may extend longitudinally for more than a centimetre. The individual elements are reticulate or

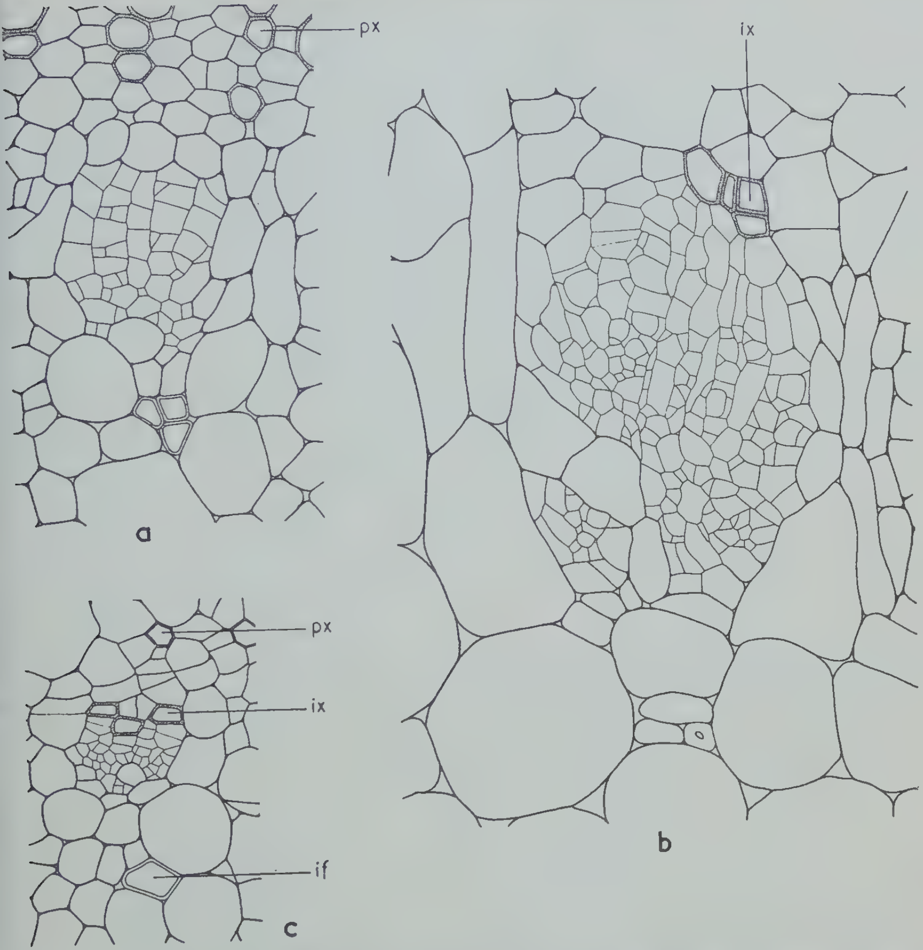

FIG. 2. (*a*) T.S. of an enlarged internal phloem strand of *Atropa* showing files of phloem cells; (*b*) T.S. of an enlarged internal phloem strand of *Lycopersicum* with a small area of internal xylem centrifugally; (*c*) T.S. of an enlarged internal phloem strand of an *Atropa* petiole showing a few cambial divisions and internal xylem elements; *if*, internal fibres; *ix*, internal xylem; *px*, protoxylem.

pitted, some two to four times as long as broad, and corresponding in their lengths (about 90 to 170 μ) to the cambial initials from which they are derived. They are not usually positioned precisely end-to-end; accordingly the end walls are often more or less oblique. It can usually be seen that these end walls have a simple perforation, so that a continuous vessel is formed.

Internal cambium and xylem can arise in petioles just as in stems. In the species used in this work the petiolar vascular tissue is in the form of an arc in transverse section, with internal phloem on its adaxial side. When petioles that have been cut into sufficiently deeply to expose the vascular tissue are grafted to stems, occasional internal phloem strands in the petiole develop

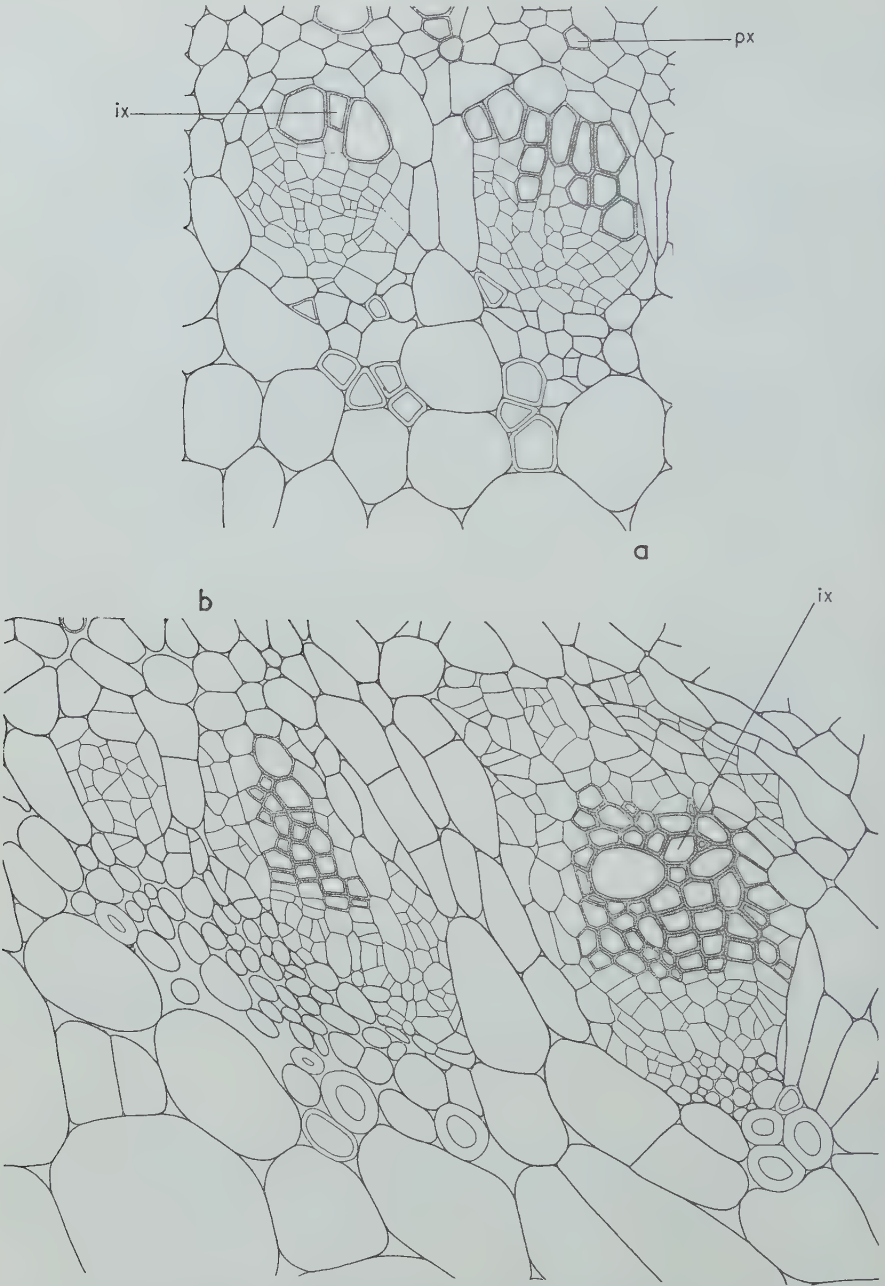

FIG. 3. (*a*) T.S. of part of an *Atropa* stem showing two enlarged internal phloem strands; (*b*) T.S. of part of a *Datura* stem showing two internal phloem strands with large amounts of internal xylem, and (left) one little-affected strand (scarline to the right of drawing); *ix*, internal xylem; *px*, protoxylem.

xylem on their abaxial side (Fig. 2*c*). Such development is, however, much less extensive in petioles than in stems.

So far as we know, traumatic formation of cambium in association with internal phloem has not previously been recorded. However, Esau (1941) describes tomato plants in which infection with curly-top virus resulted in abnormally massive internal phloem strands, and figures one such strand which is 'surrounded by hyperplastically dividing cells that form radial rows like cambium cells. On the abaxial side of the internal phloem, some of these groups of radially arranged cells have differentiated into hyperplastic sieve-tube strands'. No xylem was formed by this unilateral cambium.

It is possible that regeneration of wounds and grafts in Solanaceous plants is aided by this enlargement of their internal phloem strands. Solanaceous species are known to graft easily, and we find that their pith proliferates more actively after wounding than does the pith of species that lack internal phloem. If translocation through the internal phloem contributes towards this activity, enlargement of the phloem strands may well assist regeneration. It is more questionable whether the internal xylem is physiologically significant; however, we have found in certain cases that internal xylem strands are joined at one end to the external xylem.

The activity described in this paper tends to result in the formation of additional vascular bundles, inverted in orientation, on the inner side of the main vascular ring; and so to give the following symmetrical arrangement:

$$\text{external}\left\{\begin{array}{l}\text{phloem fibres}\\ \text{phloem}\\ \text{cambium}\\ \text{xylem}\end{array}\right.$$

$$\text{ground parenchyma}$$

$$\text{internal}\left\{\begin{array}{l}\text{xylem}\\ \text{cambium}\\ \text{phloem}\\ \text{phloem fibres}\end{array}\right.$$

Although such an arrangement does not normally occur in any Solanaceous plant, two concentric vascular cambia (the inner one inversely oriented) are known to occur in normal stems of certain species in several families, especially the Araliaceae, Campanulaceae, Compositae, and Convolvulaceae (Metcalfe and Chalk, 1950), and have also been found in stems in which a central cavity has resulted from wounding or from natural or chemically induced ring-fasciation (Worsdell, 1929; Wardlaw, 1953).

INTURNING OF EXTERNAL CAMBIUM DURING REGENERATION

When a stem is wounded in the way described above so that part of the external cambium is cut away, there tends to regenerate in the callus proliferating from the wounded surface a new vascular cambium which joins the cut ends of the original cambium, restoring a complete cambial cylinder. This occurs not merely in Solanaceous species, but generally in plants that have tissues sufficiently active to allow vascular regeneration. The Solanaceous plants used in the present work freely regenerate a cambial cylinder in this way, and transverse sections of wounded stems normally appear, after recovery, as on the right-hand side of Fig. 4*a*.

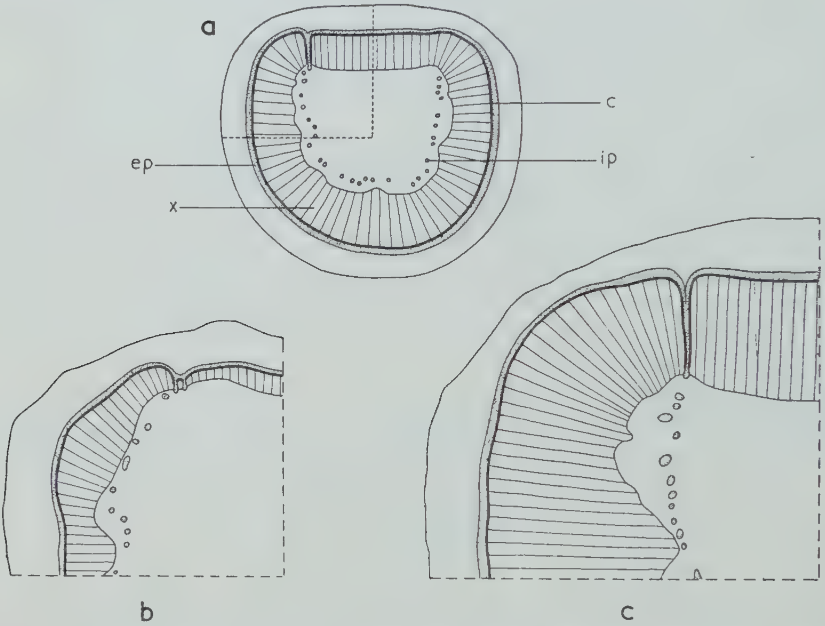

FIG. 4. (*a*) Diagrammatic T.S. of a *Datura* stem which has regenerated after wounding (not to scale); *c*, cambium; *ep*, external phloem; *ip*, internal phloem; *x*, xylem; (*b*) diagrammatic T.S. of part of a *Datura* stem corresponding to the quarter indicated by broken lines in Fig. 4*a*, 4 weeks after wounding; a pair of short, unlignified strips occurs in place of the more usual single strip; (*c*) as (*b*), 11 weeks after wounding; a single, long, unlignified strip joins the external phloem to an internal phloem strand.

However, in occasional transverse sections we find that the xylem formed from the regenerated cambium is traversed by one or (less frequently) two thin strips of unlignified tissue running radially from pith to cortex (Fig. 4*a*, left-hand side, 4*b*, 4*c*). At the outer end of each unlignified strip the external cambium turns inwards and passes down the sides of the strip. Occasional files of xylem elements derived from this inturned cambium can be seen. Towards the inner end of the strip the cells appear radially extended and there is some distortion, but it is clear that an internal phloem strand lies at the inner end of each of these structures, and, in fact, tangential sections through the regenerated wood show that these unlignified strips lie along two lines that correspond to the positions where the cut at wounding passed through the internal phloem region (Fig. 5*a*). In this plane the inturned cambium appears as an oval ring, forming phloem inwards and xylem outwards; seriation of

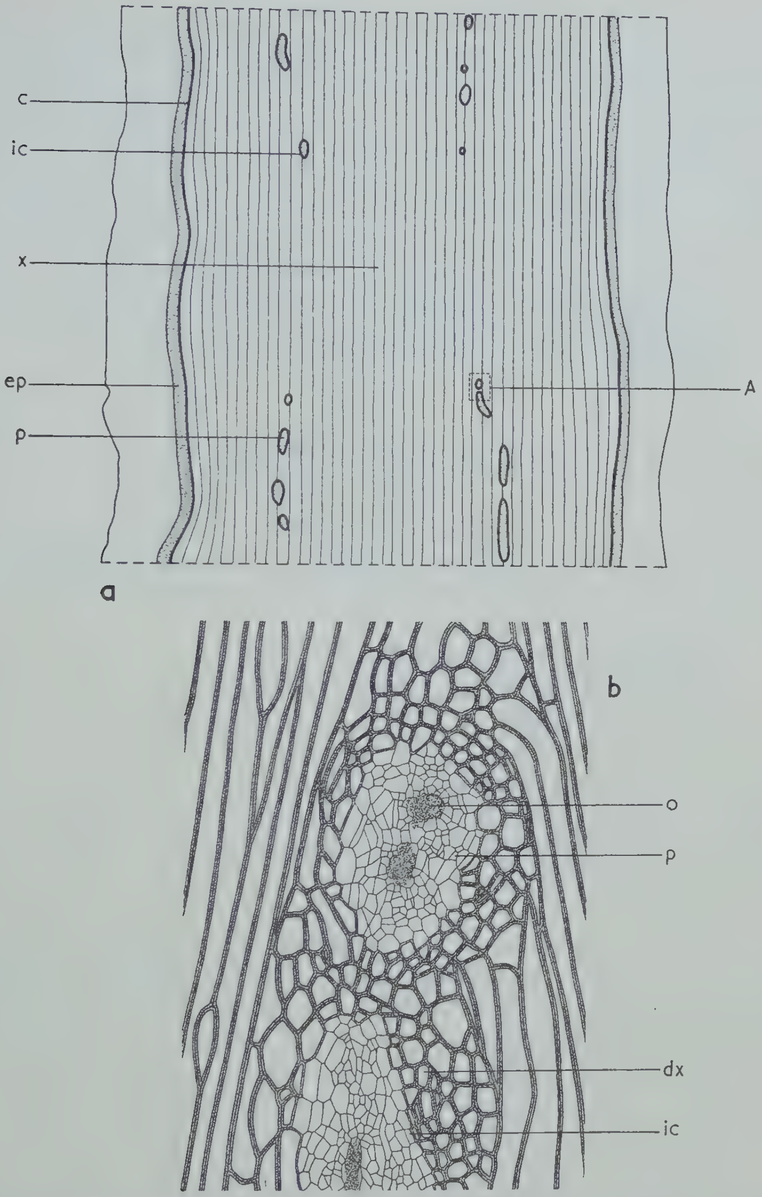

Fig. 5. (*a*) Diagrammatic tangential L.S. of a *Datura* stem, 11 weeks after wounding; *c*, cambium; *ep*, external phloem; *ic*, inturned cambium; *p*, phloem; *x*, xylem; (*b*) detail of the area marked *A* in Fig. 5*a*; *dx*, xylem derived from inturned cambium; *ic*, inturned cambium; *o*, obliterated cells; *p*, phloem.

derivatives can usually be seen (Fig. 5*b*). The size of the ring diminishes towards the centre of the stem, and this seems to be a result in part at least of continued xylem formation by the cambium; but obviously this activity is limited, since the cambial ring is enclosed within the wood. The cells within the cambial ring are small, as seen in tangential sections, and centrally there is often some densely staining material that probably represents obliterated cells.

In our experience, once this abnormal structure arises it is perpetuated; it seems that the external cambium cannot revert to its normal cylindrical form by regenerating across the narrow band of phloem included within the inturned cambium.

This abnormal structure of the cambium has the effect of uniting the external and internal phloem systems. These are in any case joined at the leaf gaps in normal stems (Artschwager, 1918; Esau, 1941), so that it is doubtful whether the abnormality is physiologically significant. Its interest is rather in relation to the theories concerning cambial regeneration that are discussed in the next section.

INTERPRETATION ACCORDING TO THEORIES OF CAMBIAL REGENERATION

We first discuss cambium formation in wounded Solanaceous stems in relation to the 'gradient induction' hypothesis proposed in an earlier paper (Warren Wilson, 1961) to define the positions in which cambia regenerate. This hypothesis states that:

(i) In undifferentiated tissue a gradient in some factor as yet unknown tends to arise perpendicular to the exposed surface.

(ii) A vascular cambium can form at only one position on this gradient, where the factor is at an appropriate level.

(iii) Cambial orientation, as regards phloem and xylem formation, is determined by the direction of the gradient.

(iv) When cambium, phloem, or xylem have differentiated, the level of the unknown factor becomes fixed around them.

(v) The gradient arising in undifferentiated tissue that immediately adjoins tissue in which a gradient is fixed tends to conform to this established gradient.

(Undifferentiated tissue may be placed so that the gradient in it is determined jointly by both surface effect (i) and by adjacent established gradient (v).)

In Fig. 6*a* and in the discussion below, the trend of the gradient is arbitrarily represented by using the symbol — for the level near the exposed surface, and + for that away from it. Cambium may then form in the plane between — and + regions, producing phloem on the — side and xylem on the + side.

In a normal stem we suppose the gradient to be present from the surface inwards, with the cambium established between the outer (—) and the inner (+) levels. In Solanaceous stems we suppose also that there are further — regions where the internal phloem has differentiated (Fig. 6*a*, lower part). Thus along a radius passing through an internal phloem group, the gradient must become reversed in direction just internal to the protoxylem. We therefore expect levels of the unknown factor to be appropriate for cambium formation between this position and the internal phloem; and because the gradient is reversed, we also expect this cambium to be inverted in orientation.

Thus expectations according to the 'gradient induction' hypothesis are in line with the observed position and orientation of internal cambia.

It must be noted that this hypothesis is concerned with the positions in which cambia may arise, but not with whether they in fact succeed in forming. It is clear that cambium formation is sometimes retarded or does not occur at all because tissue in the appropriate position is not 'competent' to form cambium. We find that cambia arise far more readily in undifferentiated than in differentiated tissue; also, we have suggested that sufficient levels of auxin and sugar may be required. In the present case we suppose that the tissues are not competent to form an internal cambium until wounding provides a stimulus, and that even then it is only in the region of the internal phloem strands that competence is achieved.

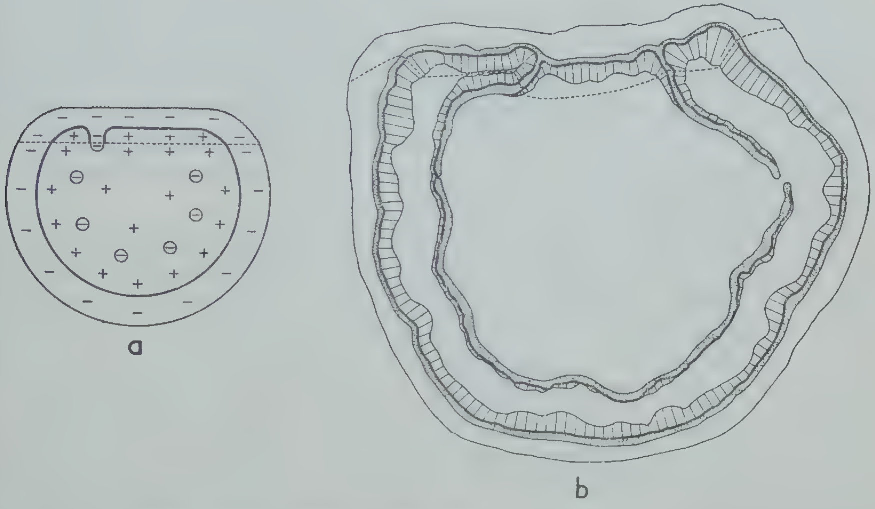

FIG. 6. (*a*) Diagram to show pattern of gradients in a Solanaceous stem which has regenerated after wounding; broken line indicates limit of regenerated tissues; thick line indicates cambium; small circles indicate internal phloem; (*b*) diagrammatic T.S. of a *Campanula* stem, 8 weeks after wounding; broken line indicates limit of regenerated tissues; for explanation see text.

To interpret the formation of an inturned cambium in a wounded Solanaceous stem, consider the expected pattern of gradients induced in the callus formed over the wounded surface (Fig. 6*a*). In the central part of the wound a gradient arises from the surface inwards and leads to the formation of a regenerated cambium roughly parallel to the surface and normally oriented. At the edges of the wound, induction of gradients by the original differentiated tissue becomes important. If no internal phloem happens to lie at the wound surface (Fig. 6*a*, right-hand side), gradients induced by the surface and by the differentiated tissue are similar and result in a simple union of the regenerating cambium with the cut end of the original cambium. If, on the other hand, an internal phloem strand occurs at the wounded surface it will induce a — region in the callus adjacent to it (Fig. 6*a*, left-hand side). Cambium, arising between + and − tissues, may now regenerate along a path which at one end joins the cut end of the original cambium and at the other end turns in towards the outer side of the internal phloem, where an internal cambium may form. The cambium regenerating across the centre of the wound ends blindly near the internal phloem, presumably because tissue on the inner side of the internal phloem is not competent to form cambium.

This interpretation of the course of cambial regeneration in relation to the internal phloem in Solanaceae is supported by observations on *Campanula pyramidalis.* In this species an internal, inverted cambium forms an almost complete ring inside the external, normally oriented cambium. When the stem is wounded, regenerating cambia usually unite the external and internal cambia (Fig. 6*b*), while the cambium regenerated in the central part of the callus becomes joined to a third, innermost cambium, which is normally oriented and is stimulated into activity by wounding.

We have discussed elsewhere (Warren Wilson, 1961) the two theories proposed by earlier workers to explain the positions of regenerating cambia. The 'free surface' theory, proposed by Bertrand and Vöchting, may be stated as follows: 'A cambium forms beneath, and generally parallel to, each natural or artificial free surface. It produces phloem on the side towards the free surface, and xylem on the opposite side.' The 'cambial ring' theory, proposed by Janse and Snow, may be stated: 'The factor which causes cambial regeneration is the interruption of the old cambial ring (as seen in transverse section), and the regenerating cambia extend from the cut edges of the ring in such a way as to form a closed ring.' These earlier theories both provide some explanation of the positions of regenerating cambia in wounded stems of normal anatomy. However, neither helps to explain the development of internal cambia in wounded Solanaceous stems; and neither explains the inturning of cambia described above, since (i) this is not appropriately related to a free surface, and (ii) it results not in closure of the cambial ring but in its permanent interruption.

We conclude, therefore, that the 'gradient induction' hypothesis is preferable to the 'free surface' and 'cambial ring' theories in that it alone helps to explain the special cambial formations that arise in wounded Solanaceous stems.

ACKNOWLEDGEMENTS

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A Study of the Nitrogen and Water Factors in Competition with *Agropyron repens* (L.) Beauv.

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With five Figures in the Text.

ABSTRACT

From a consideration of the possible relationships between the level of a nutrient in the soil, the depletion due to a competitor, and the response of a test plant, it is argued that a valid criterion for the implication of a nutrient factor in root competition is that some growth response of the test plant to competition should be less at higher levels of supply of that nutrient than at lower levels.

Young test plants of *Impatiens parviflora* were transplanted into pans of soil in which stands of *Agropyron repens* were growing. Two levels of nitrogen and two rates of watering were applied in a multifactorial design, and from half of the test plants root competition was excluded, so that their responses to root competition could be measured under the various nitrogen and watering treatments.

A preliminary experiment had shown, and the competition experiment confirmed, that the optimum nitrogen level for root growth and Net Assimilation Rate was less than the higher level employed, whereas this level was not sufficient for maximum leaf-growth. In agreement with this, the depressions of relative root growth-rate and Net Assimilation Rate caused by competition at the lower nitrogen level were significantly reduced at the higher level, but for relative leaf-growth this reduction did not reach significance. The effects of competition were reduced by the higher rate of watering, and for relative leaf growth-rate this interaction was highly significant.

It is deduced that competition from *A. repens* involves both nitrogen and water, and that water is probably the more important factor under normal conditions. There is no need to postulate any toxic root product to account for the experimental results. Compared with competition above ground, it appeared that root competition could have an effect on the relative growth-rate of *I. parviflora* about as great as a reduction of light intensity from full to 5–10 per cent. of summer daylight.

INTRODUCTION

WHERE plants grow near together it may be surmised that, besides the mutual shading above ground, there will be mutual interference with the free supply of the available nutrients and water below ground. It is this interference which is rather loosely termed root competition. The term may also include the production from roots, either by secretion or decay, of substances which are deleterious to the growth of other plants. Thus, three main factors in root competition may be recognized: competition for nutrients,

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competition for water, and the production of growth-inhibiting substances. Although previous research has covered all three of these aspects they have been treated separately, and, furthermore, there is little quantitative data suitable for comparing their relative importance, or for comparing the overall effect of root competition with, for example, shading.

There is little doubt that in particular cases nutrients, water, and toxic substances are all involved in root competition. In many instances, however, the existing experimental evidence is insufficient to support the interpretation which has been put upon it. Competition for water has been demonstrated by means of trenching experiments in woodland (i.e. experiments in which a plot of ground has been isolated from the root competition by a deep trench cut around it). Some more recent examples are described by Korstian and Coile (1938) and Shirley (1945). Where this technique has been used it would be expected that many factors other than water-supply will be affected and there is considerable evidence that this is so. Watt and Fraser (1933) and Korstian and Coile (1938) have reported changes in soil structure following trenching, and Romell (1938) has inferred from observations on fungal growth that trenching improves soil aeration and can materially affect nutrient status by causing the death of mycorrhizal fungi. Furthermore, in some cases it has been found that the artificial addition of water has produced no effect (Watt and Fraser, 1933; Kramer, Oosting, and Korstian, 1952).

Competition for nutrients has been suggested in agricultural experiments where the effect of weed removal has been compared with that of fertilizer dressings (Blackman and Templeman, 1938; Russell, Keen, and Mann, 1942), but interpretation of such experiments is rendered difficult by the fact that the shading effects were not assessed and water competition was not positively excluded. Mann and Barnes (1945, 1952, e.g.) sought to show competition for nitrogen between barley and certain weeds grown in pots. Although competition seems to have occurred, the possibility of competition for other nutrients or for light having an effect was not ruled out. More recently, in a study of competition between wheat or oats and skeleton weed (*Chondrilla juncea* L.) in Australia, Myers and Lipsett (1958) have recognized and employed the principle that removal of competition for a nutrient (nitrogen in this instance) may be expected to have a greater beneficial effect on yield on sites where the nutrient is deficient. Donald (1958), in a pot experiment intended primarily to compare competition for nutrients and for light, and to study their interactions, obtained results which also indicated that nutrient competition between the two grass species used was mainly for nitrogen.

About the production of toxic substances there is in some cases little doubt, e.g. from roots of guayule (*Parthenium argentatum*) (Bonner and Galston, 1944; Bonner, 1946). In most instances, however, the evidence rests on the results of incorporating dried or ground roots in soil to produce the toxic effect (Proebsting and Gilmore, 1940; Benedict, 1941), or of applying an extract of dried roots, as in Osvald's (1947) observations on *Agropyron repens*. Such evidence does not prove the production of toxins by living roots nor their effectiveness in nature. Experiments with the leachings of plant roots have generally not avoided possible complications due to nutrient effects, bacterial action, &c. attendant on the use of soil culture.

Bearing in mind these difficulties of interpretation it is clearly necessary to consider how competition for a given factor can best be demonstrated. It is not sufficient in the case of a nutrient factor, *n*, for example, to show that the depression of growth caused by competition can be corrected by the application of *n* to plants suffering from it. For the application of this amount of *n* to competition-free plants might have increased their growth to still higher levels and the effect of competition could therefore still be present. We may merely be compensating for deficiency of some other factors (with an independent, but similar, effect to *n*) by increasing the supply of *n*. We obviously need to consider in some detail the relations which would be expected to hold between the *n* that is applied and the effect on growth of competition for it. The relevant factors in this relationship are the effect of *n* level on growth of competing roots, the effect of these roots in removing *n* from the soil, and the effect of *n* level on growth of the test plant. Of these, the first was as far as possible eliminated in the experiment which is the subject of this paper, by allowing stands of the competitor to become well established before any differential treatments were introduced, by following competition effects for a relatively short time after they were introduced, and by choosing a nutrient having a relatively small effect on root development (see p. 121). We shall consequently be concerned chiefly with the other two factors.

First, let us suppose that the response of the test plant is directly proportional to *n* level (Fig. 1*a*). If now the competing roots were to remove a constant amount of *n*, the effect of competition would be independent of *n* level. If, on the other hand, competing roots removed a constant fraction of the available *n*, the effect of competition should increase with the *n* level. In fact our initial proposition is unlikely to be true and the growth response of the test plant is more probably curved, with the effect of increments of *n* falling off at higher levels (Figs. 1*b* and *c*). Here, if competition removed a constant amount of *n*, its effect would clearly decrease as the *n* level rises. On the other hand, if a constant fraction were removed, the change in the competition effect would depend on the form of the growth-response curve of the test plant. It is easy to show that if the growth happened to be proportional to log *n*, the competition effect would be constant (Fig. 1*b*). But if the response of the plant approximates to a rectangular hyperbola of the Michaelis type, as it might well do, the effect of competition should decrease as higher levels of *n* are reached (Fig. 1*c*). It seems improbable that competing roots will continue removing an amount of *n* proportional to the amount present at very high *n* levels and this fall-off would further diminish the effect of competition in the upper parts of the range (Fig. 1*d*). From these arguments it would appear that if root competition causes less depression of growth at high levels of a given nutrient factor than at low levels, there is good evidence for the implication of that factor in the root competition.

The same argument would also apply to water. In terms of the statistical analysis of the effects of treatments in a multifactorial design, this means that we should look for a significant positive interaction between the effects of competition and of the factor concerned. At the same time it should be appreciated that the absence of any such interaction does not disprove the implication of this factor.

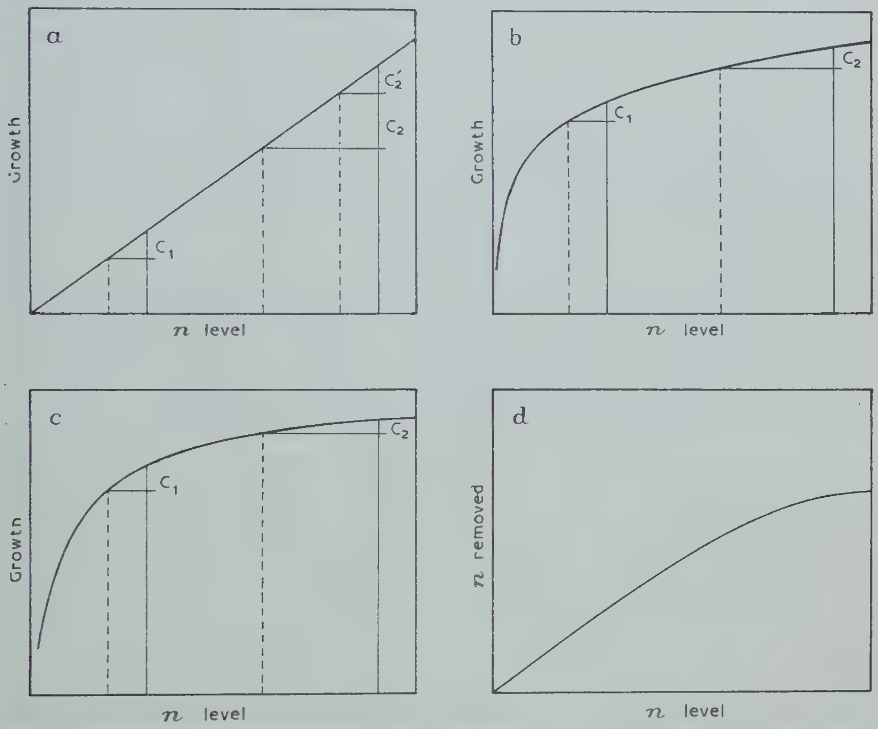

FIG. 1. Possible effects of competition for a nutrient, n, on growth with (*a*) linear response of the test plant to n level, (*b*) response curve logarithmic, (*c*) response curve a rectangular hyperbola, (*d*) suggested relation between n level in soil and amount of n removed by a competitor. Solid vertical lines represent growth at original n levels, dotted lines at level to which n is assumed to be reduced by competition.

C_1: effect of competition on growth at lower n level,
C_2: at higher level, C_2': effect at higher level if n removed were constant.

Considering briefly the effect of higher n levels on the growth of the competing roots, this could best be taken into account by its effect on the amount of the available n that these roots would remove on account of their increased size. In general, it can be seen that such augmented growth should tend to increase the effect of competition at high n levels, and hence would not invalidate conclusions based on an observed decrease under these conditions. In the particular case of the experiment described below, while the response of *Agropyron repens* to nitrogen was not measured, it is known that nitrogen has comparatively little effect on root growth generally. It is conceivable that copious watering could be harmful to competing *A. repens* roots because of leaching or water-logging, but it is considered unlikely that any such action materially affected the results of this experiment. The technique was designed to minimize the effects of treatments on the *A. repens* and although it was not possible to check this quantitatively, visual observation suggested that any such effects were not appreciable.

In this discussion we have been considering one set of competing roots as lowering the level of nutrient available for the other. For a complete analysis of a developing competition situation it would be necessary to take into account the mutual effects of all competitors, but at the present stage the above concept is of considerable value because of the simplification in the experimental approach to which it leads. From a practical point of view (e.g. competition between weeds and crops) it is also satisfactory, and it could have possibilities where one competitor is clearly dominant (root competition from trees) or where for any reason measurements on both species in a society cannot be made.

Based on these principles a technique has been developed in which suitable test plants are transplanted into the soil in which the competing species is already established. The effect of root competition on the test plants is measured when different levels of water or nutrients are supplied, and it is then possible to infer the probable action of the competitor on the root environment. The choice of species for the present experiment was largely one of convenience. The test plant, *Impatiens parviflora* D.C., by reason of its habit and because it is an annual, is in many ways very suitable for growth-analysis work. A full account of the species has been published by Coombe (1956). *Agropyron repens* (L.) Beauv. (couch grass) was chosen for investigation because of its reputation as a strong competitor and because it is said to produce a toxic root secretion (Osvald, 1947). In a pilot experiment it was found to produce a satisfactory response in *I. parviflora*. With the resources available it was possible to combine the investigations of two potential factors in root competition in a multifactorial design. In order to make its scope as wide as possible, water and a nutrient (nitrogen) were selected as the two factors for the first experiment, nitrogen being chosen because it was considered likely to produce the most marked responses in the test plant.

PRELIMINARY INVESTIGATION ON THE RESPONSE OF *IMPATIENS PARVIFLORA* TO NITROGEN

Before setting up an experiment in which the interaction between root competition and nitrogen effects on the growth of *Impatiens parviflora* could be observed, it was necessary to know something of the form of response to nitrogen shown by this species. A preliminary experiment was therefore carried out for this purpose.

Plants of *I. parviflora* were grown in pots in essentially the same manner as in the competition experiment described later (p. 122) as regards the transplanting technique and the soils used. Growth was measured over a 3-week period in soil containing no additional nitrogen and in the same enriched by additions of approximately 50 and 200 p.p.m. nitrogen (as ammonium sulphate). These levels were chosen to obtain a nearly maximal response between the lowest two, and it was hoped that it would then be shown that there was little further improvement in growth between the middle and highest levels. Two levels of phosphorus were also used at the lowest nitrogen level to test for effects of this element on growth. Growth analysis was carried out, using the dry-weight and leaf-area data obtained from harvests after 2 and 3 weeks' growth (West, Briggs, and Kidd, 1920). From the results of this experiment it appeared in the case of some growth characteristics, e.g. root growth and rate of dry-weight increase / unit leaf area (Unit Leaf Rate, ULR, of West, Briggs, and Kidd, 1920; Net Assimilation Rate, NAR, of Gregory, 1926), that although small additions of nitrogen produced increases, these were reduced when more than about 50 p.p.m. of nitrogen were added to the soil. On the other hand, the leaf growth, and hence the Leaf Area Ratio (LAR), continued to increase up to the highest nitrogen level used. It would thus be impracticable in a competition experiment to use a level of nitrogen so high that depletion by competing roots had no effect at all on the growth of *I. parviflora*. The slight falls in root dry-weight growth and Net Assimilation Rate (NAR) which were found between the middle and highest nitrogen treatments, although not statistically significant, are interesting because they appeared to be repeated in the subsequent competition experiment. Because of the fall in NAR at higher nitrogen levels, overall Relative Growth Rate (RGR) reached a maximum with between 50 and 200 p.p.m. added nitrogen.

These results are in agreement with the observations of other workers. For example, Ballard and Petrie (1936) found that in both wheat and Sudan grass (*Andropogon sudanensis*) root growth was depressed at high nitrogen levels (even at 30 p.p.m.) in sand culture and actually found leaf size depressed in the early stages of growth. McLean (1957) reports that for a number of crops grown in gravel culture the roots were hardly affected by nitrogen level and in oats they tended to be reduced. Watson (1947) gives results for fertilizer experiments with mangolds which make it fairly clear that, although leaf area is increased by the addition of nitrogen, there is little effect on NAR.

Rather less could be deduced about the effects of phosphorus, but it did appear that the resultant increases in growth were general to all parts of the plant. In an experiment on phosphorus as a factor in root competition it may, therefore, be more practicable than in an experiment on nitrogen to use a level of the nutrient so high that competition for it ceased to be effective.

COMPETITION EXPERIMENT

Experimental Technique

The statistical design of the experiment followed the principles already laid down. For convenience in referring to the various treatment combinations, they are designated by combinations of the symbols *n*, *w*, and *c*, which indicate the use of the higher level of nitrogen, watering, and competition respectively, their absence indicating the lower level (following the system of Yates, 1937). Thus, '*n*' indicates the combination of high nitrogen, low watering, and no competition; '*nc*' indicates high nitrogen, low watering, and competition, &c. The symbol (1) is used for the treatment combination with all factors at the lower level. Capital letters are used to designate the main effects of treatments, thus: *N*, *W*, *C*; and interactions are designated by the combination of the appropriate capitals, thus: *NW*, *NC*, *NWC*, &c.

In order to keep block size as small as possible the main effects *N* and *W*, and the interactions *NW* and *NWC*, were each confounded in one pair of blocks, so that the completed design consisted of eight blocks of four soil treatments in four replicates of two blocks each. Provision was made for an initial harvest and two subsequent harvests at weekly intervals.

The plants were grown in a mixture of 1 part Kettering loam to 4 parts silver sand, to which was added a basal fertilizer dressing consisting of $CaHPO_4$, 8·25 g., K_2SO_4, 22·4 g., $MgSO_4.7H_2O$, 3 g., and NaCl, 3 g. per bushel (approx. 54 kg. dry weight) of soil. These quantities of phosphorus and potassium were equivalent to about 80 p.p.m. of P_2O_5 and 200 p.p.m. of K_2O respectively on a dry-weight basis. (An exception to this was that the *A. repens* was raised in soil to which only 5 p.p.m. P_2O_5 was added because at the time it was thought that phosphorus might be included in the differential treatments.) The soil was contained in unglazed pans, 11 inches (28 cm.) in diameter and 5½ inches (14 cm.) deep which were plunged to the rim in the ground at about 3 ft. (1 m.) spacing.

Agropyron repens was grown from rhizome sections planted round the outer part of the pan of soil. After some 8 weeks' growth the pans were rearranged to give as uniform growth of grass as possible within each block. Single plants of *Impatiens parviflora* were then transplanted into the centre of each pan at the beginning of the experiment proper as described below (p. 124). Two levels of root competition were achieved by excluding competing roots from half of the *I. parviflora* plants by growing them in waxed 5-inch pots plunged in the soil in the pans (Fig. 2). So that this could be done without undue damage to the grass roots, a spare 5-inch pot was placed in the centre of each pan from the start, and the *A. repens* planted in the annular space around it. At the same time as the dummy pots were replaced by waxed pots containing the experimental soil (with high or low nitrogen levels, see below) for the without-competition plants, the dummies were removed and replaced by the appropriate experimental soil *without* any waxed pot for with-competition plants. This was done 1 week before the transplanting of the test plants so that development of competing roots in the central ball of 'experimental' soil could take place, while reducing to a practicable minimum the effect of differential nitrogen treatment on the *A. repens*. These arrangements were made to ensure that above-ground effects were as uniform as possible for all treatments, although it was subsequently shown that any effects of shading by *A. repens* tops were slight.

The nitrogen levels which were used were those present in the basic mixture (low nitrogen) and the same increased by the addition of ammonium sulphate equivalent to approximately 100 p.p.m. nitrogen (25·36 g. per bushel). This amount was judged from the results of the preliminary experiment to be not greatly above the optimum for root growth and NAR, at the same time giving a possibility of observing a reduction in the effect of competition on leaf-growth. Analysis of soil samples taken at the commencement of the experiment proper gave values for total available (ammonium and nitrate) nitrogen of 95 p.p.m. in the high-nitrogen and 24 p.p.m. in the low-nitrogen soil. It would appear that already a considerable part of the added nitrogen was no longer available.

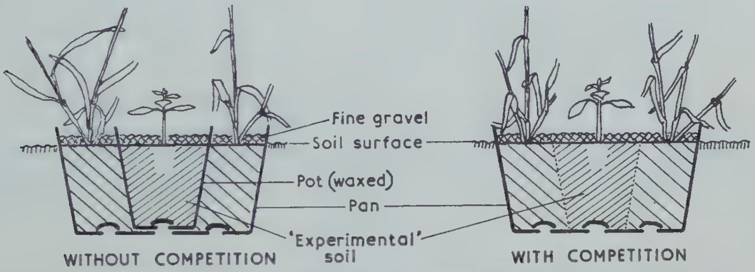

FIG. 2. Arrangement of pots for growing *Impatiens parviflora* with and without root competition.

The lower level of water-supply was obtained by natural rainfall, supplemented on two occasions when it was feared that wilting was causing a risk of leaf abscission (the 3rd and 12th days after transplanting) by small amounts of tap water. The higher level of watering was achieved by bringing the soil up to field capacity every other day. This was done by adding a measured excess of water to four test pans and subtracting the amount which percolated through in about 45 minutes to determine the volume to be applied to each watered pan in the experiment. Tap-water was used and was sprayed evenly over the whole surface of the soil in the pan so that *I. parviflora* plants growing without competition received only an amount proportional to the area of the central 5-inch pot. It is not possible to define exactly what the differences in water availability between the two treatments were. By the end of the 3rd day plants in the *c* and *nc* combinations showed signs of wilting, but in the night of the 4th day rainfall of 34 mm. was recorded and about 1 litre of water passed through each pan—presumably more through the watered pans. Thereafter the unwatered pans slowly dried out until wilting was again incipient on the 12th day.

Impatiens parviflora plants were grown from seed in standard loam–sand mixture in thumb pots (i.e. 4×4 cm.). At 3 weeks, 8 sets of about 15 uniform plants each were selected and after a further week's growth each set was reduced to 10 closely matched plants. Four additional plants were taken for the initial harvest to increase slightly the precision of regression estimates (see p. 128 below). After the second matching the sets were allocated to blocks, and the plants within sets to treatments and harvests, using tables of random permutations. The lengths and breadths of leaves, and stem height and thickness (just below the cotyledonary node) were then recorded for each plant. All cotyledons were removed at this stage, some already showed signs of senescence; the plants had six or seven leaves apparent.

The *I. parviflora* plants, except for the initial harvest, were transplanted into the centre of the pans with *A. repens* on day 0 (4 July 1956). Experiment had previously shown that this treatment did not result in any appreciable check in growth. A layer of fine gravel was spread over the soil in each pan to reduce rain splashing of soil and all pans given a uniform watering. At the first and subsequent harvests leaves were printed on to photographic paper for planimetering, and roots, stems, and leaves dried separately. On the day before the second harvest (day 6) all the *I. parviflora* plants were measured as before. This was not done again before the third harvest, but otherwise the same procedures were used for second and third harvests (on days 7 and 14). It was found fairly easy in the case of plants grown with competition to separate *I. parviflora* roots one at a time from the mass of *A. repens* with very little breakage after most of the soil had been removed by gentle agitation under water. Root washing was later completed with a fine spray.

To provide a rough check that there were no marked differences in the form of leaf-area growth curves between the various treatments, measurements were made of the leaf sizes of one plant from each treatment combination at intervals of 2 days throughout the experiment. The third-harvest plants of the replicates in which the *NWC* interaction was confounded were used for this purpose. Included in the final harvest were 2 plants grown with each of the four treatment combinations not involving competition which had been grown in pans without the central waxed pot and with no *A. repens* planted. No significant differences from plants grown in a central pot to exclude competition were found, which indicates that there were no adverse effects on growth arising from confinement of the roots to a 5-inch pot, and also that any effects of the aerial parts of *A. repens* were slight.

STATISTICAL AND COMPUTATIONAL PROCEDURES AND RESULTS

The leaf areas and root, stem, and leaf dry weights of each plant were transformed to logarithms for analysis of variance because the effects of treatments on any size variate, z, were expected to be proportional to z. In this way the effects of treatments on the relative growth-rates of the plants were studied. To bring in both second- and third-harvest results in order to improve estimates of error, analyses were performed using values of

$$\log z_1 + 2 \log z_2 \qquad (a)$$

for pairs of matched plants, where z_t is the value of z measured on a plant after t weeks. The use of this function, suggested by Professor H. E. Daniels, depends on the effects of treatments on $\log z$ being proportional to t. Whether there were significant departures from this condition was tested by analysing values of a second function

$$\log z_2 - 2 \log z_1 \tag{b}$$

for which all the treatment effects (given as natural logarithms in Table 2) should be zero. This procedure avoids a difficulty which would arise if second- and third-harvest data were analysed together directly, with harvest time treated as an additional factor at two levels. A treatment producing a specific increase in relative growth-rate will result in a difference in $\log z$ between treated and untreated plants which increases linearly with time. There would, therefore, be confusion between interactions with time of this sort and variation with time of treatment effects on relative growth-rates.

TABLES 1 AND 2

Main Effects and Interactions of Treatments on Weighted Mean Relative Growth-rates (above) and Non-linearity with Time of $\log_e$ *of Primary Data (below)*

	Dry weight				
Effect	Total	Root	Stem	Leaf	Leaf Area
Mean	0·621	0·661	0·535	0·609	0·747
N′	0·150***	0·056**	0·132***	0·272***	0·272***
W′	0·020	0·022	0·010	0·034	0·066*
NW′	0·026	0·018	0·038	0·026	0·006
C	−0·182***	−0·060***	−0·184***	−0·328***	−0·342***
NC	0·036*	0·070***	0·036	0·028	0·040
WC	0·052**	0·034*	0·034	0·090***	0·110***
NWC′	−0·034	−0·038*	−0·024	−0·042	−0·038
S.E.	0·0154	0·0140	0·0190	0·0199	0·0258
S.E.′	0·0178	0·0162	0·0222	0·0229	0·0298
Mean	0·124	0·094	0·246	0·053	−0·049
N′	0·098	0·128	0·254*	−0·030	−0·058
W′	−0·040	0·012	0·012	−0·048	−0·084
NW′	0·020	0·088	0·048	0·022	−0·202*
C	−0·116	−0·182*	−0·136	−0·024	0·074
NC	−0·124	−0·014	−0·136	−0·0178	−0·372***
WC	0·006	0·036	0·034	−0·068	0·040
NWC′	0·012	0·086	0·028	−0·126	−0·090
S.E.	0·0716	0·0800	0·0980	0·1044	0·0810
S.E.′	0·0826	0·0924	0·1132	0·1206	0·0936

Note: Asterisks indicate conventional levels of significance.
*, $P < 0·05$; **, $P < 0·01$; ***, $P < 0·001$.
Confounded main effects and interactions indicated by ′.
S.E. and S.E.′ refer to unconfounded and confounded effects respectively.

The function (*a*) gives efficient estimates of differences in the regression coefficient of $\log z$ on t (equivalent to relative growth-rate) due to treatments. [All the regression lines of $\log z$ on t for different treatments must be assumed to have a common point at time t_0 because the plants were initially alike except for block differences and random sampling errors. If the value of log z at this point is taken as log z_0 the estimate of the slope of such regression lines obtained by the method of least squares is

$$\frac{\sum(t-t_0)(\log z_t-\log z_0)}{\sum(t-t_0)^2}$$

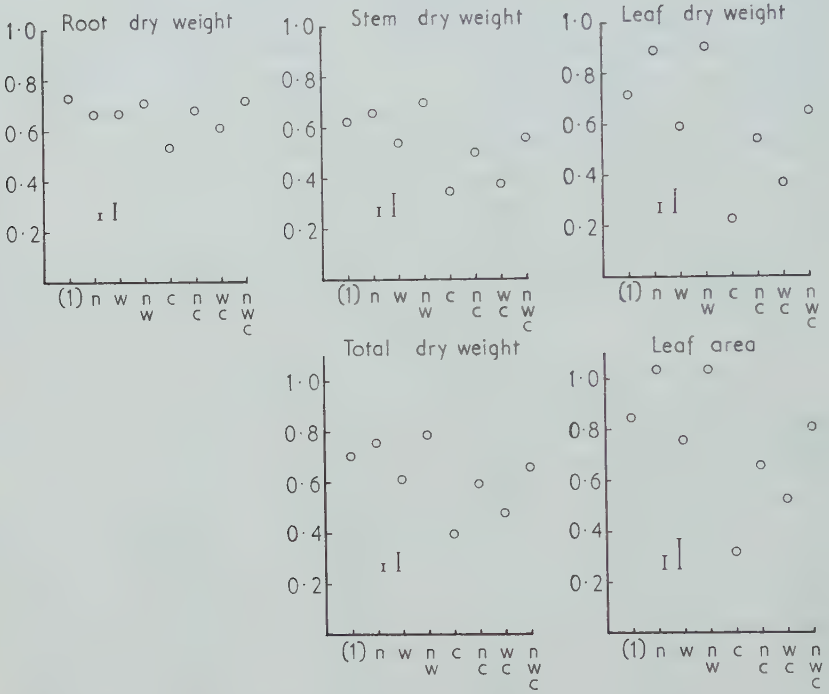

FIG. 3. Weighted mean relative growth-rates per week for the 2-week period for each treatment combination. Vertical lines show standard error of mean (left) and least difference significant at the 5 per cent. level. (Note: because of the confounding, standard errors of comparisons between adjusted treatment means depend on the particular comparison concerned; the least differences significant at the 5 per cent. level indicated are those for comparisons with the highest standard errors, the lowest are about 0·93 as great.)

(cf. Snedecor, 1946, § 6. 3). Substituting known values of t in the present instance this becomes

$$\frac{[\log z_1-\log z_0]+2[\log z_2-\log z_0]}{5}$$

and since all the main effects and interactions are estimated from differences between pairs of treatment combinations, log z_0 will be eliminated.] The treatment effects for the analyses of (a), expressed as relative growth-rate by converting to natural logarithms and dividing by 5, are given in Table 1;

estimates of the treatment means (adjusted to allow for the confounding) were calculated as

$$\tfrac{1}{5}(\log_e z_1+2\log_e z_2)-\tfrac{3}{5}(\log_e z_0)$$

and are shown graphically in Fig. 3. Estimates of the treatment means of relative growth-rates in the separate weeks shown in Fig. 4 were calculated from the adjusted treatment means of $(\log z_1+2\log z_2)$ and $(\log z_2-2\log z_1)$ thus:

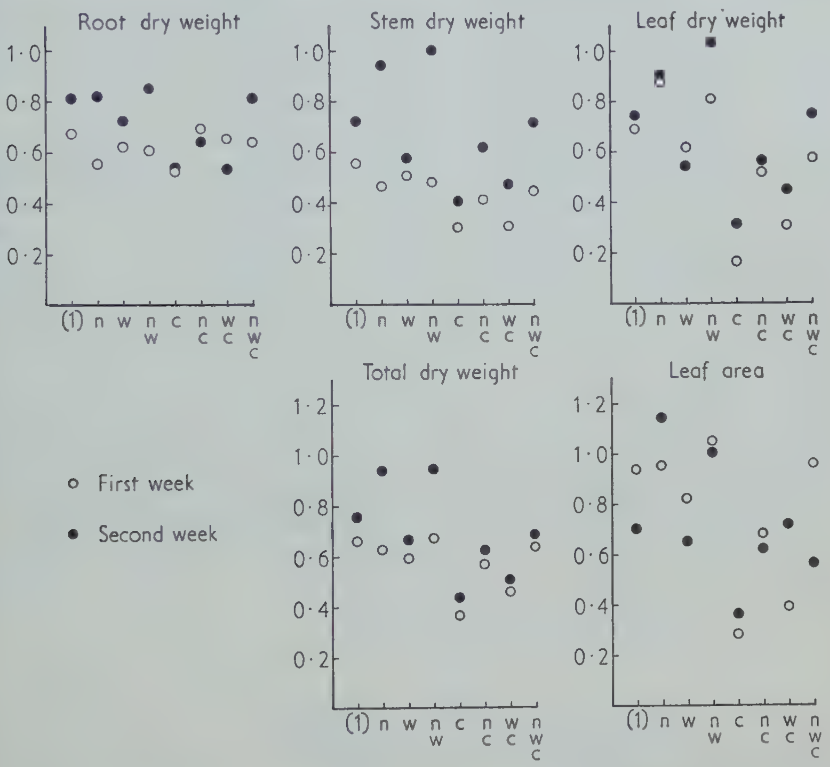

FIG. 4. Relative growth-rates per week in the separate weeks for each treatment combination.

first week: $\tfrac{1}{5}[(\log_e z_1+2\log_e z_2)-2(\log_e z_2-2\log_e z_1)-5\log_e z_0]$

second week: $\tfrac{1}{5}[(\log_e z_1+2\log_e z_2)+3(\log_e z_2-2\log_e z_1)]$.

No useful increase in precision of the analyses of primary data was obtained by covariance analysis, using the linear measurements of the plants at the time of the initial harvest as independent variables, and the procedure was therefore not generally applied.

Growth analysis (West, Briggs, and Kidd, 1920) was carried out using the dry-weight and leaf-area data to obtain further evidence of the factors involved in root competition and to investigate its effects on metabolism. Dry weights and leaf areas of plants at the beginning of each week were estimated by means of regressions of total dry weight and leaf area on functions of the linear measurements of the plants, made, as already described, while they were still growing. The regression equations were computed from the linear measurements and the dry weights and leaf areas of plants in the previous harvest. This procedure is similar to that which was used by Goodall (1945, 1950) and other workers and leads to important reductions in error over the alternative method in which the size of one plant which is actually harvested is used as an estimate of the size of a plant still growing, with which it was matched by eye at the commencement of the experiment.

Leaf areas were estimated from the linear regression of area on the sum of the products of leaf lengths and breadths, $\sum (L \times Br)$. Since there were no noticeable differences in leaf shape, the data for initial and second harvests were combined in calculating the regression equation, and initial leaf areas of both second- and third-harvest plants were estimated from it. The initial dry weights of the second-harvest plants were estimated using the multiple regression of dry weights of first-harvest plants on leaf $\sum (L \times Br)$ and stem length×thickness², $L \times D^2$. The total dry weights of third-harvest plants at the time of the second harvest were obtained from multiple regressions of the logarithms of total dry weights of second-harvest plants on the logarithms of their leaf $\sum (L \times Br)$ and stem $L \times D^2$. In this case it was necessary to carry out a full analysis of variance and covariance because there were differences between treatments in the relation of dry weight to linear dimensions.

Growth-analysis data were then calculated for each plant using the following formulae:

$$RGR = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

$$NAR = \frac{W_2 - W_1}{t_2 - t_1} \frac{\log_e A_2 - \log_e A_1}{A_2 - A_1}$$

$$LAR = \frac{A_2 - A_1}{\log_e A_2 - \log_e A_1} \frac{\log_e W_2 - \log_e W_1}{W_2 - W_1},$$

where W_1 and A_1 are the estimates of total dry weight and leaf area at time t_1 and W_2 and A_2 are actual values at time t_2. The individual values were treated by analysis of variance, but in the case of these and other derived data the considerations differ somewhat from those affecting the statistical analysis of the primary data. Since the effects of treatments were not expected to increase linearly with time, it was decided to analyse the results of each week or harvest separately, and in general the data were not transformed. The main effects of treatments and their interactions are given for growth-analysis variates in Table 3 and adjusted treatment means are shown in Fig. 5.

Table 4 presents a summary of the effects of nitrogen, water, and competition on all the variates discussed, as affected by the *NC* and *WC* interactions.

TABLE 3

Main Effects and Interactions of Treatments on Principal Derived Quantities

Effect	First week RGR per wk.	First week LAR cm.^{2}/mg.	First week NAR mg./cm.^{2}/wk.	Second week RGR per wk.	Second week LAR cm.^{2}/mg.	Second week NAR mg./cm.^{2}/wk.
Mean	0·623	0·1259	4·94	0·718	0·1399	5·01
N′	0·098***	0·0136***	0·26	0·204***	0·0256***	0·65***
W′	0·026	0·0048	0·04	−0·001	0·0070	−0·21
NW′	0·022	0·0042	−0·02	−0·006	−0·0032	0·03
C	−0·135***	−0·0168***	−0·42**	−0·366***	−0·0356***	−1·43***
NC	0·018	0·0064*	−0·09	0·111***	0·0002	1·00***
WC	0·046**	0·0024	0·30*	0·040*	0·0126**	−0·12
NWC′	−0·017	0·0012	−0·19	0·042*	0·0018	0·14
S.E.	0·0148	0·0023	0·136	0·0164	0·00328	0·122
S.E.′	0·0171	0·0027	0·158	0·0190	0·00380	0·142

* See note to Tables 1 and 2.

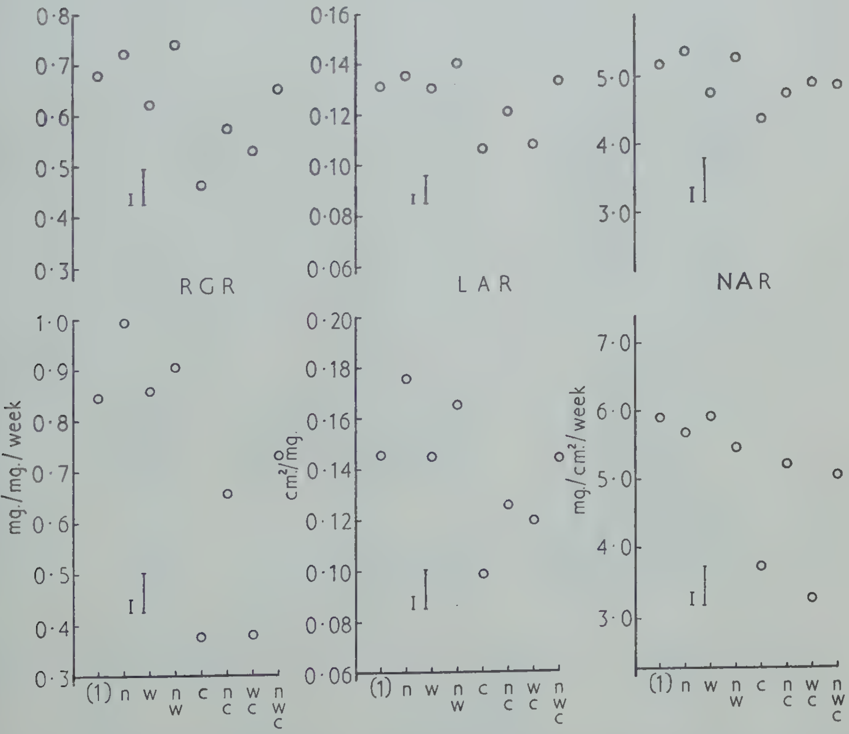

FIG. 5. Growth analysis data, relative growth-rate RGR, leaf area ratio, LAR, and net assimilation rate, NAR; means for each treatment combination, 1st-week values above and 2nd-week values below. S.E.M. and least difference significant at the 5 per cent. level shown as in Fig. 3. The vertical scales for each week are arranged with origins all at the same level. (See note to Fig. 3.)

TABLE 4

Summary of Treatment Effects as Influenced by NC *and* WC *Interactions*

	Averages of low and high watering				Averages of low and high nitrogen			
	Effect of nitrogen		Effect of competition		Effect of watering		Effect of competition	
	Without *c*	With *c*	Low *n*	High *n*	Without *c*	With *c*	Low *w*	High *w*
Weighted mean relative growth-rates per week								
Total dry weight .	0·114***	0·186***	−0·218***	−0·146***	−0·032	0·072*	−0·234***	−0·130***
Root dry weight .	−0·014	0·126***	−0·130***	0·010	−0·012	0·056*	−0·094***	−0·026
Stem dry weight .	0·096**	0·168***	−0·220***	−0·148***	−0·012	0·022	−0·218***	−0·150***
Leaf dry weight .	0·244***	0·300***	−0·356***	−0·300***	−0·056	0·124***	−0·418***	−0·238***
Leaf area . .	0·232***	0·312***	−0·382***	−0·302***	−0·044	0·176***	−0·452***	−0·232***
Derived Data				*First week*				
RGR per week .	0·080**	0·116***	−0·153***	−0·117***	−0·020	0·073**	−0·181***	−0·088***
LAR cm.²/mg. .	0·0072	0·0200***	−0·0232***	−0·0104***	0·0024	0·0072	−0·0192***	−0·0144***
NAR mg./cm.²/wk. .	0·35	0·17	−0·51*	−0·32	−0·26	0·33	−0·71**	−0·12
				Second week				
RGR per week .	0·093**	0·315***	−0·477***	−0·355***	−0·041	0·039	−0·406***	−0·326***
LAR cm.²/mg. .	0·0254***	0·0258***	−0·0358***	−0·0354***	−0·0056	0·0196**	−0·0482***	−0·0230***
NAR mg./cm.²/wk. .	−0·35	1·65***	−2·43***	−0·43*	−0·32	−0·09	−1·31***	−1·55***

DISCUSSION

Before proceeding with the discussion of the effects of the experimental treatments on the test plants it will be convenient to consider whether there is evidence of any differences in the main effects of treatments and their interactions between the 1st week of the experiment and the 2nd. Where such differences are absent we can legitimately base our conclusions on the average effects of treatments for the whole period of the experiment. It is clear from Table 3 and Fig. 5 that the LAR and NAR responses to treatments were not the same in the 2 weeks. Furthermore, from Table 2 which can be examined in conjunction with the estimates of relative growth-rates in the separate weeks shown in Fig. 4, it will be seen that in 4 cases there were significant departures from proportionality with the time among the effects of treatments on the logarithms of dry weights and leaf areas. It is possible to suggest tentative explanations for some of these differences between weeks.

External factors which might have been responsible were chiefly climatic and included a heavy rainfall (34 mm.) which occurred during the 4th night, and generally more overcast and humid weather in the 2nd week. This may have accounted for the absence of any significant *WC* interactions among the 2nd week NAR values, but it is worth noticing that there were signs of *WC* interactions among other data, for example, relative leaf growth-rates. On the other hand, differences may be accounted for by intrinsic changes in the experimental material. One would expect that increases in the effects of competition would be found in the 2nd week as the plants grew larger and exploited more of the available soil. A less obvious explanation for the generally greater effects of treatments on NAR, &c. in the 2nd week is the fact that during the 1st week the plants would have been largely dependent on metabolic machinery which was laid down before transplanting and therefore not affected by treatments, particularly where growth was slow as in the *c* and *wc* combinations. Also the LAR values were weekly means and the apparent size of treatment effects on this ratio in the 1st week would therefore have been reduced by the initial uniformity of the plants.

Of the four cases in which the effect of treatments on relative growth-rates of plant parts differed significantly between the 2 weeks, only for the *NC* interaction on leaf-area growth was the significance at all high. This extreme difference was due to unexpectedly high growth-rates under the *wc* treatment combination and low growth-rates under the *nwc* combination in the 2nd week. No satisfactory explanation for this has been found, but it may be relevant that similarly high *wc*, and low *nwc* values were found for the 2nd week among total nitrogen and nitrogen uptake-rate data. Of the other three cases where the differences between weeks reached significance, that of the *NW* interaction on the leaf-area growth-rate is judged from an inspection of the growth-rate estimates for the separate weeks to be fortuitous, but the greater effect of nitrogen on stem growth and the greater effect of competition on root growth in the 2nd week might both be explained if there had been some leaching of nitrogen by the heavy rainfall of the 4th night. These cases in which treatment effects were found to differ between weeks must be borne in mind during the following discussion, and it will to some extent be necessary to consider the results for each week separately, particularly for the derived data of NAR, LAR, &c. It is clear, however, that at least for the relative growth-rates of the plant parts we are justified in proceeding on the assumption of constancy with time so far as the bulk of the effects are concerned.

Competition for Nitrogen and Water

The most striking result of the experiment is that although competition as a rule caused a very large depression of relative growth-rates, the depression was less with high nitrogen or water-supply in nearly all cases. The *NC* interactions on relative root growth-rate, and on NAR in the 2nd week, were both highly significant. In the absence of added nitrogen, competition produced a significant decrease in root growth and a large decrease in NAR, but where nitrogen had been added there was no decrease in root growth and only a small (just significant) decrease in NAR. Taken in conjunction with previous knowledge of the responses of root growth and NAR to nitrogen (p. 121), the simplest and most likely explanation of these observations is that root competition was acting by removing nitrogen from the soil. The *WC* interactions were usually less marked than these *NC* interactions, but they appear to have been more generally shown by the growth of all parts and by leaves in particular. It is considered unlikely that the effects of watering could have been due to the washing out of any toxic substance, because only amounts estimated to be necessary to bring the soil up to field capacity were added, and the *WC* interaction is therefore attributed to depletion of water in the soil by root competition. This conclusion is supported by the fact that plants of *I. parviflora* receiving *c* and *nc* treatments were found to be partially wilted on several occasions during the course of the experiment.

It is possible from the data to make some comparison of the relative importance of the nitrogen and water factors in root competition. From the RGR's it would seem that for the plant as a whole the importance of water competition was slightly greater, and might have appeared more so had it not been thought necessary to add small amounts of water to pots receiving the low-water treatment to prevent leaf abscission. Competition for water had much more effect than competition for nitrogen so far as limiting leaf growth was concerned, but somewhat less effect than competition for nitrogen in limiting root growth. From the RGR's for the separate weeks (Fig. 5) it becomes clear that this overall importance of water depletion arose mainly from its effect in the 1st week, in the 2nd week it was of less consequence than competition for nitrogen, probably as a result of the changed climatic conditions. It is also apparent that in both weeks the more important factor in competition affected RGR through its effect on NAR.

Growth Inhibitors

It has so far been inferred that root competition from *Agropyron repens* was acting through both water and nitrogen depletion and it can reasonably be supposed that other nutrients would also be implicated if their availability was limited. There is, however, little or no evidence for the occurrence of any toxic secretion. As already stated, it is regarded as unlikely that the *WC* interaction was due to leaching out of a toxin. If there had been associated with competition a reduction of the size of a part or of some other characteristic of the plant, even when supplies of water and nutrients were more than adequate for its maximum development, this might have been attributed to some third factor, such as a toxin. Among those characteristics (e.g. root growth and NAR) for which these conditions were fulfilled, however, no significant depression was caused by competition when both nitrogen and water were supplied at the higher level. Where competition did produce a significant effect with this treatment combination (e.g. on leaf growth), other evidence indicated that nitrogen was a controlling factor even at the higher level used. There is thus little need to invoke a toxin to explain the results of the present experiment. This does not, of course, disprove the presence of a toxin, which may be effective on a different species or under different experimental conditions. Indeed, it may be quite difficult to rule out toxin action in an experiment of this type. It has been suggested, for example, that the observed *WC* or *NC* interactions could be caused by a toxin, if it became effective only at low levels of water or nitrogen supply. An increase in activity of the toxin at low water levels would be consistent with its dependence on concentration as well as quantity. A greater effect at low nitrogen levels might be expected if the toxin acted as a competitive inhibitor for nitrogen uptake, but it would seem more likely that it would act non-competitively partly to inactivate the uptake mechanism, and its effect on uptake should then be proportional to the uptake rate. The growth response would be similar to that due to a depletion of soil nitrogen by a constant fraction, and it would evidently be difficult to distinguish between toxin action and direct competition for nitrogen.

Effect of Nitrogen and Water

Besides its main purpose of providing evidence for the mechanism of root competition, this experiment also produced useful information on the responses of *I. parviflora* to nitrogen and water-supply.

Nitrogen generally increased growth, more so in the presence of competition, but high nitrogen in the absence of competition actually produced a small decrease in relative root growth-rate. Although this decrease was not significant in itself, it supports the similar observation made in the preliminary experiment (see above, p. 121). Nitrogen increased the LAR through its greater effect on leaf growth than on stem and root growth. It had little effect on NAR in the 1st week, but in the 2nd week, although its effect on NAR in the absence of competition was small, where competition was present nitrogen produced a very marked increase. This suggests that the NAR response of the plant to nitrogen status becomes appreciable only at very low nitrogen levels. There was again an indication of a fall in NAR produced by excessive nitrogen applications (cf. p. 121), and if this effect is genuine, it may possibly be the result of self-shading or it might be caused by increases in respiration, such as have been reported by White (1936) to account for a similar NAR response in *Lemna minor*.

Watering alone produced small depressions in the growth of all parts of the plants, and although individually these were for the most part hardly significant, this fact is of some interest. Since leaching was avoided as far as possible, the depression, if real, should more probably be attributed to some other consequence of the treatment, such as dilution of the soil solution or poor aeration. With competition present, watering resulted in increased growth of all plant parts, but especially of leaves. As a result of this there was an increase in LAR which was significant in the 2nd week. An effect of watering on NAR in the presence of competition might have been expected, at least in the 1st week, but although there was an increase, it did not reach significance. It seems likely that this, and the non-significance of the watering effect on LAR in the 1st week, was due to the initial uniformity of the material.

Root Competition and Shading

The data which have been obtained in the present experiment permit a comparison to be made between the effects of root competition on *Impatiens parviflora* and the effects of shading which have been studied by other workers (Coombe, 1952; A. P. Hughes, personal communications). Coombe compared the light intensities at three situations in *Ulmus diversifolia-Quercus robur—Acer campestre* woodland when the canopy was just expanding at the end of May: (*a*) in a clearing; (*b*) under the canopy, but with no understory development; and (*c*) under *Corylus avellana*. Compared with the clearing intensity, the two more shaded situations had intensities of 20 and 7 per cent. respectively (measurements with a selenium cell and a green filter). Actual values of NAR obtained for one period in the clearing and under these two degrees of shade were 3·9, 1·3, and 0·5 mg./cm.^{2}/week respectively. The reduction in NAR brought about by shading is, however, compensated up to a point by an increase in LAR, so that the RGR is actually increased by moderate degrees of shading. If we assume that light intensity in Coombe's clearing was 60 per cent. of full daylight, his results on this point are in fair agreement with those of Hughes, who used artificial shades, within the limits of the rather large error which is inevitable in this sort of measurement, and indicate reductions in RGR of about 30 and 70 per cent. of the full daylight value at 12 and 4 per cent. of full daylight respectively. The species is clearly very shade-tolerant.

If we take the effect of root competition without added nitrogen in the present experiment as giving the best indication of its possible effects in nature, and if we concentrate on 2nd-week values, as being less affected by initial uniformity of material, we find that NAR was reduced by about 40 per cent. In contrast to shading, however, this treatment also reduced LAR and the depression of RGR was about 56 per cent., an amount which is comparable with that due to fairly deep shade. Had water deficiency been greater the effect on growth-rate of root competition might well have been more.

It does not follow that tree roots would have as large a competitive effect as those of *Agropyron repens* in this experiment, nor can it be assumed that the root competition response would be as great in shade as in the open. On the other hand, we have already seen how Hughes has found that owing to the increase of LAR, moderate degrees of shading increase the RGR of *I. parviflora*, and it may therefore be that under such conditions root competition would be a much more restrictive factor than shading.

SUMMARY

1. If the effects of one plant on another growing with it can be reduced by adding a nutrient factor (or water), this is evidence that competition for that factor is involved in the interaction between them. Such a reduction will most probably be obtained where the relationship between plant response and level of the factor concerned is appreciably curved over the range used, with the effect of equal increments of the factor falling off as its level rises.

2. The relative root growth-rate and NAR of *Impatiens parviflora* showed suitably curvilinear responses to nitrogen, although its relative leaf growth-rate response was substantially linear over the range of N levels used.

3. The effects of competition were observed on young test plants of *I. parviflora* when transplanted into established stands of *Agropyron repens*, with nitrogen supplied at two levels and with soil moisture allowed to fall to wilting point or maintained near field capacity. Phosphorus and potassium were supplied at high levels.

4. The higher nitrogen level significantly reduced the effects of competition on relative root growth-rate and NAR of *I. parviflora*, but not its effects on relative leaf growth-rate. Competition effects on both root and leaf relative growth-rates were significantly reduced by the higher rate of water-supply.

5. Competition here appears to involve both nitrogen and water, and it seems likely that water is the more important factor under normal conditions. There was no evidence of toxin secretion from underground parts of *A. repens*.

6. High nitrogen levels appeared to depress root growth and NAR of *I. parviflora*, and very low nitrogen levels resulting from competition also decreased NAR considerably.

7. Root competition may have an effect on relative growth-rate of *I. parviflora* comparable with that due to a reduction in light intensity from full to 5 to 10 per cent. of summer daylight.

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Correlations between Growth and Flowering in *Chenopodium amaranticolor*

I. Initiation of Leaf and Bud Primordia

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With three Figures in the Text

ABSTRACT

Vegetative plants of *Chenopodium amaranticolor* were induced to flower by exposure to 2, 6 or continuous short days (SDs) and the effect of such treatments on organogenesis at the apex of the main stem followed by means of dissections.

The most outstanding responses to SD treatment were (1) an immediate elongation of the apex, (2) a stimulation of the rate of initiation of leaf primordia, and (3) a promotion of the rate of initiation of axillary bud primordia. In response to as few as 2 SDs, the rate of initiation of leaf primordia increased from 0·47 to a maximum of 3·70 per day and the rate of initiation of axillary bud primordia immediately increased from 0·47 to 1·35 per day.

Precocious initiation of axillary bud primordia led to the formation of double ridges. The results indicate double ridges to be homologous with vegetative axillary buds; although they normally developed into reproductive tissues, they passed through a period of vegetative growth following minimal induction to flowering by exposure to 2 SDs.

The rate and degree of flowering were highest in plants which received the longest period of SDs, but the differences in final flowering response were greater than the differences between the initial responses at the apices. The effect of SDs was thus not confined to an initial stimulation of organogenesis; a prolonged exposure to SDs must have enhanced the subsequent development of double ridges into flower primordia.

The results are discussed in relation to previous findings and the general conclusion drawn that the initiation of double ridges is very widely accompanied by a stimulation of apical growth. It is suggested that inductive conditions remove a general growth inhibition and that the resultant stimulation of apical growth might lead to the initiation of double ridges.

INTRODUCTION

NUMEROUS investigations have been made in the past into the influence of external factors, such as photoperiod, temperature and applied growth substances, on flowering. In many of these cases the degree of flowering induced by the treatment given has been recorded in terms of the rate of growth of the inflorescence, e.g. the time taken to the appearance of macroscopic flower buds. But such measures of flowering record only the later stages of flower development and fail to describe the initial response of the plant to an inductive stimulus. For a complete understanding of the flowering process a knowledge of these initial apical changes is essential. Wardlaw (1959) has

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stated: 'there can be no true understanding of morphogenesis unless there is precise observation of the size, shape and relationships in time and space, of nascent organs in embryonic regions. . . . Without this exact preliminary information, there can be no proper formulation of the relevant physiological problems.'

The amount of such preliminary information describing the changes at the apex which accompany the transition to flowering is very slight, but the results of one or two investigations do point to the considerable benefit to be gained from observation of the morphogenetic changes occurring at this stage. Thus from the results published by Philipson, and reviewed by him in 1949, it is apparent that one of the outstanding characteristics of an apex during its transition to the reproductive condition is the precocious formation of axillary bud primordia. This stimulation leads to the formation of the double ridges described by Purvis and Gregory (1937). Whether these precociously formed buds are homologous with vegetative axillary buds and whether conditions which promote the formation of double ridges also stimulate the initiation of vegetative buds are clearly questions of some importance when considered in relation to the changes occurring within an apex during its transition to the floral state.

The rate of organogenesis at the reproductive apex has not been the subject of detailed anatomical and morphological investigations in the past. Clearly, however, a knowledge of the rates of processes occurring at the apex during this stage of development could be of fundamental importance to our understanding of the reproductive process. Schwabe (1959) has estimated the rate of bract initiation in the inflorescence of *Chrysanthemum* to be nearly three per hour compared with a basic rate of foliage leaf initiation of nearer three per week. How closely this high rate of initiation is related to the transition to the reproductive phase, how rapidly the rate of initiation changes during this period, and whether similar changes occur in plants in which the transition from the formation of foliage leaf primordia to the initiation of bract primordia is more gradual are some of the questions raised by his observations.

The present study was undertaken in an attempt to provide preliminary answers to some of these questions. *Chenopodium amaranticolor*, a short-day plant, was selected as a species in which there is a gradual transition from the development of foliage leaves to bracts at the onset of sexual reproduction and an experiment was set up to investigate the influence of three intensities of flower-inducing stimuli on the rates of apical organogenesis, particularly with reference to the initiation of leaf and bud primordia and the relationship between the two. At the same time, records were made of the differences in stem elongation and leaf size and shape brought about by the various treatments and these are reported in Part II.

MATERIAL AND METHODS

Seeds of *Chenopodium amaranticolor* were sown at Palmerston North in November 1959. After germination, seedlings were potted individually into a

1:1 sand/loam soil mixture and grown in 16-hour photoperiods. No additional nutrients were supplied to the plants initially but from the 68th day onwards each pot received 50 ml. Hoagland's solution three times a week. When the plants had reached an 'adjusted' age of 26 days, they were divided into four groups to receive the following treatments:

A. Continuous 16-hour photoperiods (LD)—(36 plants)
B. Continuous 8-hour photoperiods (SD) —(14 plants)
C. Two SDs followed by a return to LDs —(36 plants)
D. Six SDs followed by a return to LDs —(36 plants)

These four treatments were designed to bring about responses ranging from the maintenance of the vegetative state in group A to the induction of maximal flowering in group B.

Throughout the experiment, plants were grown in a glasshouse in which temperatures were maintained at 25±3°C. during the photoperiod and 19±2°C. during the nyctoperiod. Plants growing in 16-hour photoperiods received natural daylight supplemented in the morning and evening by light at an intensity of approximately 500 foot-candles at the plant apices from 200 watt 'Sieray' mercury-vapour lamps ballasted with a tungsten filament. Plants receiving 8-hour photoperiods were placed in a light-proof box at 4 p.m. and removed at 8 a.m. daily. Both long and short daylength treatments were given in the same glasshouse and the temperatures inside and outside the box differed by no more than ±1° C. at any time during the night.

Within each of the four groups, 8 plants were allowed to grow for a period of several months and measurements of the growth of their leaves, stems, and axillary buds were made at intervals during this time. Data relating to flower positions and the times taken to reach anthesis were also collected from these plants.

The remaining 90 plants were grown for examination and measurement of the apical region of the stem under a stereoscopic dissecting microscope. These dissections provided data on the rates of initiation of leaf and bud primordia and their rates of growth during the primordial stage. The first samples dissected in groups A, C, and D each consisted of 10 plants and the other 10 samples were each made up of 6 plants.

A Developmental Index of Plant Age

One of the main purposes of the present investigation was to determine the effect of various daylength treatments on the initiation of leaf and bud primordia. It was therefore necessary to have a fairly accurate knowledge of the numbers of primordia present on each plant immediately preceding the start of such treatments as a basis from which to assess the numbers of new leaf and bud primordia initiated. In an attempt to satisfy this requirement and to reduce the variability of the samples studied, a developmental index of plant age was devised, similar in concept to that previously adopted for studies of *Xanthium* by Erickson and Michelini (1957).

This index was based on the finding that, for plants of similar vigour, the size of a rapidly expanding leaf at a given node is closely correlated with the number of leaf primordia at the apex. In a preliminary investigation immediately preceding the start of the main experiment, and run under the same conditions, it was found that the length of leaf 9 (i.e. the ninth leaf from the base of the main stem) increased from 22 to 29 mm. in 24 hours, and that when its length fell between these limits the number of leaves and leaf primordia on the main stem ranged from 24 to 27 with a mean of 25·67±0·44.

The average time taken from germination to this stage was 26 days and plants in which the length of leaf 9 lay between 22 and 29 mm. were therefore defined as having reached an 'adjusted' age of 26 days. Dissection of plants which had reached a true chronological age of 26 days showed the number of leaves and leaf primordia on the main stem to range from 22 to 28.

The length of leaf 9 was thus used as a developmental index of plant age throughout the present investigation. This leaf was measured daily between 8 and 10 a.m. and individual plants of groups B, C, and D were placed in SDs as soon as they had attained an 'adjusted' age of 26 days. Comparisons with plants in group A were then made on the basis of the same developmental scale.

RESULTS

Initial Changes Occurring at the Apex

Rate of initiation of leaf primordia. Between germination and the time at which plants reached an 'adjusted' age of 26 days, an average of 25·9 leaf primordia were initiated at the apex of the main stem of each plant. After the start of experimental treatments the number of leaves and leaf primordia present on the main stems of samples of plants from each of the four groups was estimated at intervals over a period of 16 days by dissection of the apical regions. Results of these dissections are shown in Fig. 1 and Table 1.

Immediately after the start of treatment, the most noticeable change at the apex of plants receiving SDs was a marked elongation similar to that which has been frequently observed at the transition to flowering in many other plants (Philipson, 1949).

Following this period of elongation there was a very pronounced stimulation of the initiation of leaf primordia in groups receiving SDs. Throughout the period of 16 days during which dissections were made, the number of leaf primordia formed on the plants in the LD group lay between 0·35 and 0·66 per day.

In the continuous and 6-SD groups, on the other hand, the average rate increased from zero on the 1st and 2nd days to 2·35 on the 3rd and 4th and on to 3·50 on the 5th and 6th days after the start of treatment. Beyond this time the rate of initiation decreased and eventually returned to zero as the apices were replaced by terminal flowers which prevented further initiation of leaf primordia. In the group maintained in continuous SDs the terminal flowers had been initiated by the time the plants were 36 days old, but transfer to

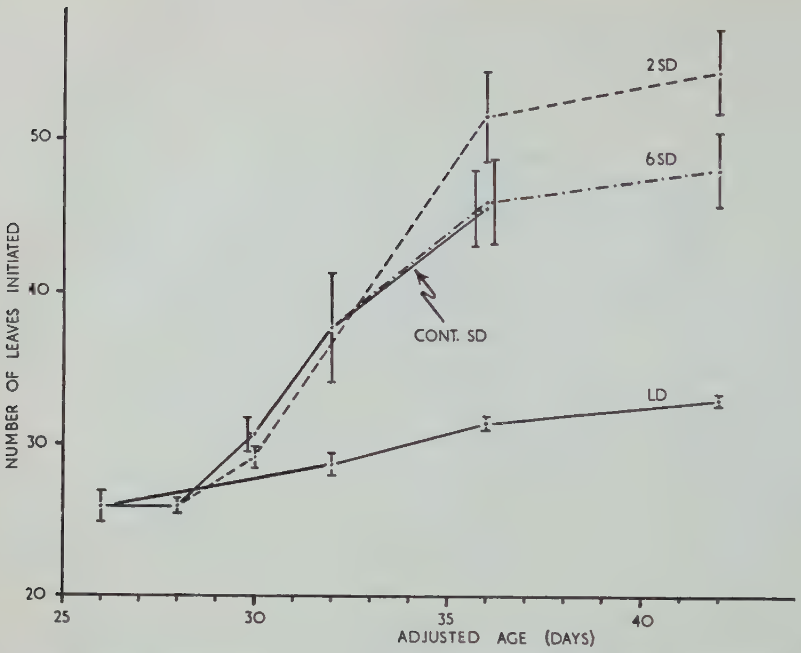

FIG. 1. The effect of exposure to 0, 2, 6, or continuous SDs on the rate of initiation of leaf primordia at the apex of the main stem. Vertical bars represent ± twice the standard error for each point.

TABLE 1

The Average Number of Leaf Primordia Formed per Day during Successive Periods throughout the 16 days following the Start of Inductive Treatment on day 26

<table>
<tr><th rowspan="2">Treatment:</th><th colspan="5">'Adjusted' age (days)</th></tr>
<tr><th>26–28</th><th>28–30</th><th>30–32</th><th>32–36</th><th>36–42</th></tr>
<tr><td>LD</td><td colspan="3">←0·46→</td><td>0·66</td><td>0·35</td></tr>
<tr><td>2 SD</td><td>0·00</td><td>1·60</td><td colspan="2">←3·70→</td><td>0·47</td></tr>
<tr><td>6 SD</td><td>0·00</td><td>2·35</td><td>3·50</td><td>2·05</td><td>0·50</td></tr>
<tr><td>Continuous SD</td><td>0·00</td><td>2·35</td><td>3·50</td><td>1·93</td><td>—</td></tr>
</table>

LDs after 6 SDs delayed their formation for a few days. The perianth segments of these flowers appeared to form in phyllotactic sequence with the leaf primordia of the main stem and were indistinguishable from them at first. They have therefore been included as leaf primordia for the purpose of estimating leaf formation rates. In those plants maintained in SDs without dissection, an average of 42·37 nodes was finally recorded. The final node number observed in the dissected plants averaged 45·33±1·25. Subtraction of the number of perianth segments in a terminal flower (five) from this figure gave an average node number of 40·33 which is quite close to the figure of 42·37 obtained for the undissected plants.

The rate of initiation of leaf primordia on plants exposed to only 2 SDs followed the same pattern as on those which received 6 or more SDs. The return to LDs, however, slightly decreased the stimulation during the 3rd and 4th days in comparison with that recorded in the other SD groups; the rate of initiation over these 2 days was only 1·6 per day in the 2-SD group compared with 2·35 in the 6-SD and continuous-SD groups. From the 4th to the 10th day after the start of SD treatment the average rate of initiation of leaf primordia was 3·7 per day and significantly higher than that observed on plants which received more than 2 SDs. This difference was probably directly related to the presence of terminal flowers in the latter plants; such flowers, which prevent further initiation of leaf primordia, were not detected in the 2-SD plants at this stage. After the 10th day, the rate of initiation of primordia in the 2-SD group dropped again to the vegetative rate of 0·47 per day.

Initiation of axillary bud primordia. The course of initiation of axillary bud primordia at the apex of the main stem in response to the various treatments was followed by dissections made at intervals after treatments began and the results so obtained are shown in Fig. 2. All axillary buds and bud primordia were recorded which were visible at a magnification of ×80 after the removal of subtending leaf primordia.

Immediately prior to the start of experimental treatments, axillary buds and bud primordia were visible at an average of 18·2 nodes on the main stem. On the average the 7·7 youngest leaf primordia, of the 25·9 which had been initiated by this time, subtended no visible axillary bud primordia.

In plants maintained in LDs, the number of budless leaf primordia remained fairly constant and initiation of primordial buds and leaves proceeded at the same rate, i.e. at an average of 0·47 per day during the 16 days following the start of experimental treatments.

Exposure of plants to SDs, however, induced a pronounced stimulation of initiation of axillary bud primordia relative to leaf primordia. During the first 2 SDs an average of 2·70 axillary buds was initiated compared with an estimated number of 0·92 in the LD controls and, as a result, initiation of leaf primordia having ceased during this period, the number of leaf primordia without visible axillary bud primordia decreased to 5·0. Exposure to another 2 SDs increased the rate of initiation of bud primordia still further and by the end of the 4th SD a bud was visible in the axil of every leaf.

In plants returned to LDs after receiving 2 SDs, the rate of initiation of bud primordia during the 3rd and 4th days was slightly lower, and from the start of SD treatment to the end of the second LD these plants produced 6·8 axillary bud primordia and the number of budless leaf primordia was reduced to 3·9. No plant which received even the slightest degree of SD induction possessed any budless nodes by the end of the 6th day after the start of treatment.

Initiation of bud primordia recorded in Fig. 2 can be divided into two types.

During the first 2 SDs, bud primordia were initiated in the axils of leaf primordia which were present but budless at the start of treatment. These were similar to the bud primordia initiated during vegetative growth and may be termed 'vegetative' primordia. During and after the 3rd and 4th SDs, bud primordia which formed in the axils of the leaf primordia above node 26 on the main stem appeared at almost the same time as their subtending leaf primordia. The units so formed, each consisting of a leaf primordium together with its axillary 'reproductive' bud, were morphologically equivalent to the double ridges of rye described by Purvis and Gregory (1937).

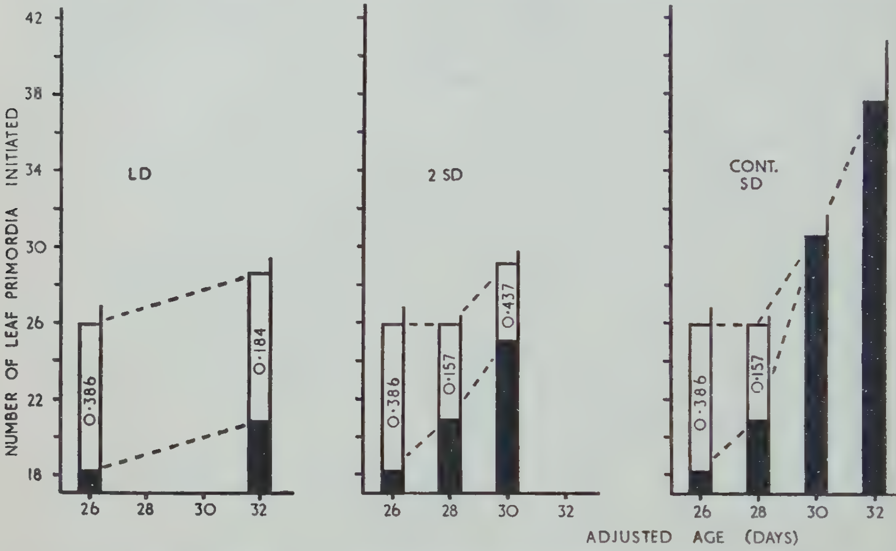

FIG. 2. The effect of treatment on the rates of initiation of leaf and axillary bud primordia at the apex of the main stem. The total column height shows the average number of leaves and leaf primordia initiated (vertical bars represent twice the standard error) and the average number of axillary buds and bud primordia is indicated by the shaded portion of each column. The number of budless leaf primordia is represented by the unshaded part of each column (enclosed figures indicate twice the standard error).

Development of axillary bud primordia. The growth of axillary bud primordia after their initiation is shown diagrammatically in Fig. 3. From this it is apparent that SD treatments not only stimulated their initiation but also enhanced subsequent bud growth. It is also clear from this figure that further bud growth and development in the 2-SD group was stimulated less than in plants exposed to 6 or more SDs.

By day 30, sufficient bud growth to allow the initiation of leaf primordia had occurred only at node 20 and below in the 2-SD group, whereas in plants receiving more SDs leaf primordia were present on axillary buds two nodes higher.

The initiation of axillary bud primordia upon the axillary buds of the main stem was similarly stimulated much less in the 2-SD group than in plants exposed to more SDs. Results of dissections made on day 36 show that in the

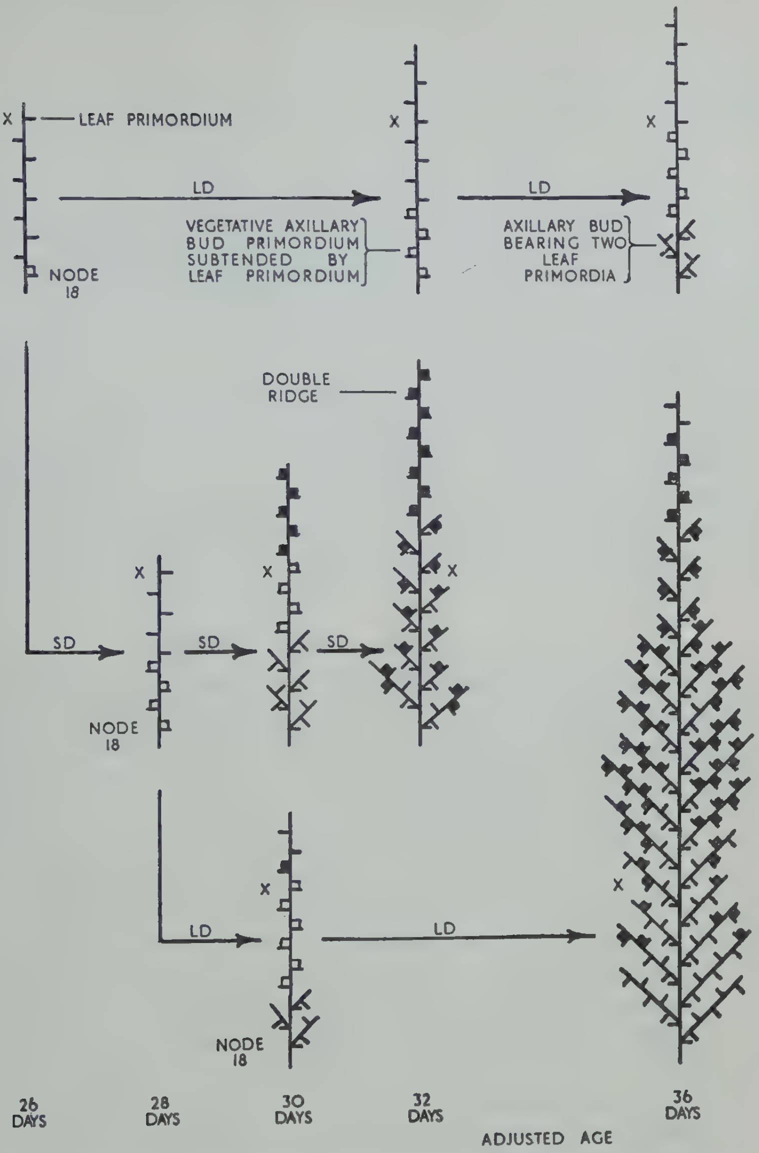

Fig. 3. Diagram to illustrate the average effect of SD and LD treatments on the initiation and development of axillary buds. X= node 26.

2-SD group the initiation of double ridges on the axillary buds was delayed below node 38. On the buds at such lower nodes, double ridges were not initiated until after the formation of at least one preceding leaf primordium. 'Vegetative' bud primordia were initiated in the axils of these preceding leaf primordia. At node 38 and above, the formation of double ridges on axillary buds occurred without the prior initiation of budless leaf primordia.

In plants receiving more than 2 SDs, however, dissections made as early as day 32 show that immediate initiation of double ridges occurred on axillary buds at all main stem nodes from 25 upwards.

Although it was possible to distinguish two types of axillary bud primordia on the main stem on the basis of the time of their initiation relative to that of their subtending leaf primordia, the clear differences between the two types were only maintained during subsequent growth and development in plants exposed to 6 or more SDs. In these plants, double ridges initiated on the main stem (at node 26 and above) developed into axillary buds which immediately produced more double ridges, and 'vegetative' bud primordia initiated on the main stem (below node 26) initiated leaf primordia before double ridges were formed.

In the 2-SD group, there was no sharp difference of development above and below node 26; instead, there was a progressive difference in axillary bud growth and development from node to node. From Fig. 3 it is clear that double ridges initiated on the main stem above node 26 in this group developed similarly to the 'vegetative' bud primordia initiated in plants receiving 6 SDs. The existence of any fundamental difference between the two types thus seems unlikely.

The Effect of Treatment on Flower Development

The final flowering response of the plants was recorded as a means of comparing the initial apical responses to the treatments given with the final degree of flowering induced by them.

In plants such as soybean and *Pisum sativum*, the degree of flowering can be expressed in terms of the lowest node at which flowers are visible on the main stem. In these species the first flowers formed are not borne on lateral branches and such a measure is quite a satisfactory one. In *Chenopodium*, on the other hand, no flowers are borne directly on the main stem and all lateral branches eventually bear flowers. A measure of flower position can be made, however, by taking the leaf growth on these branches into account. With strong flowering, leaf growth on lateral branches is very strongly inhibited and bract formation is thereby induced. With weaker induction the inhibition of leaf growth is less pronounced and bract formation is delayed (see Part II). As a measure of flowering in the present investigation, the 'lowest flowering node' was considered to be the lowest node at which the axillary branch bore no foliage leaves. On this basis the 'lowest flowering node' averaged $17{\cdot}75 \pm 0{\cdot}49$ in the plants exposed to continuous SDs and $23{\cdot}62 \pm 1{\cdot}05$ in the 6-SD group. By the end of the experiment axillary branches were visible at an average of 40·25 nodes in the 2-SD group and all were leafy at this stage (when the plants were 133 days old) even though flower primordia had been initiated. LD controls remained completely vegetative.

The average time taken to the onset of anthesis, as judged by the first day of anther dehiscence on each plant was similarly dependent on the degree of inductive treatment. Plants which were not removed from SDs reached anthesis 41·5 days after the start of SD treatment and in those which received 6 SDs anthesis occurred after 85·0 days. Plants exposed to 2 SDs had not reached anthesis by the end of the experiment, 107 days after the start of inductive treatment. Continued observation of these plants beyond this time showed them to reach anthesis eventually.

The results obtained using both these measures of flowering response were very similar: continuous SDs gave the strongest induction to flower and 2 SDs the weakest. The difference between the continuous-SD and 6-SD groups, however, was far more marked in these measurements than appeared to be the case from examination of the apices soon after treatments began. Dissections of the apical regions at this stage, the results of which are shown in Figs. 1 and 2, failed to reveal much difference between the continuous- and 6-SD groups. This observation can be explained by postulating that all stages of reproductive development well beyond the stage of initiation of double ridges are also promoted by SDs. On this basis the formation of double ridges would have been maximally induced by exposure to 6 SDs but their development into flower primordia could have been further promoted by exposure to an extended period of SD treatment.

DISCUSSION

Past observations of the vegetative changes correlated with the onset of flowering have generally led to the conclusion that vegetative growth is decreased during the reproductive phase. While this is, indeed, often true for the later stages of flowering, results of the present investigation point to the occurrence of increased growth of the tissues at the stem apex concomitant with the early stages of flower initiation.

The response of the apex of *Chenopodium* to photoperiodic induction can be considered from three separate, though probably interdependent, angles: elongation of the apical dome, initiation of leaf primordia, and initiation of axillary bud primordia. Certain facets of the general stimulation of growth have been reported from time to time in the past but no complete picture has emerged of the changes occurring at the apex during the transition from a vegetative to a reproductive state. It is now possible to assemble the existing rather fragmentary data on the framework provided by the present results and to consider the sequence of events as a whole.

(*a*) *Apical elongation.* The first noticeable response of *Chenopodium* to SD treatment was a rapid elongation of the apical meristematic dome above the youngest leaf primordium and this was accompanied by an apparent temporary cessation of the initiation of leaf primordia lasting for 2 days. Similar rapid elongation and/or swelling of stem apices has been observed by Philipson (1949) and others to accompany the onset of reproductive development in the several plants, with inflorescences ranging from capitula to simple racemes, which they have studied. A concomitant cessation of leaf initiation was also noted in *Bellis* and was regarded by Philipson (1946) as a direct result of the rapid increase in apical diameter.

(*b*) *Initiation of leaf primordia.* Six days after the onset of SD conditions, the rate of initiation of leaf primordia in *Chenopodium* was nearly eight times as high as had occurred preceding inductive treatment. In vegetative plants growing in LDs the plastochron was approximately 2·2 days, whereas after 6 SDs it had decreased to approximately 6·9 hours. This reduction is comparable with that found between foliage leaves and bract primordia in *Chrysanthemum.* Schwabe (1959) found that under his experimental conditions the rate of foliage leaf formation on vegetative *Chrysanthemum* plants was 2·2 to 3·2 per week but following the initiation of an inflorescence receptacle the rate of bract formation was estimated to be 2·7 per day.

The relationship between the rate of initiation of leaf primordia and the onset of inductive conditions is clearly very close. In plants with inflorescences as widely different as those of *Chenopodium* and *Chrysanthemum*, the transition to the reproductive phase is accompanied by a very pronounced stimulation of the initiation of leaf primordia. In *Chenopodium* this stimulation is little less after exposure to 2 SDs than that induced by a much longer period of photoperiodic treatment, and it therefore seems that the promotion is more closely related to flowering than to some other process influenced by daylength. It is probable that conditions brought about by inductive treatment and active in directing morphogenetic processes towards sexual reproduction also lead to the promotion of the initiation of leaf primordia in a wide range of plants.

Under natural conditions, in which abrupt daylength changes do not occur, any leaf primordium initiated as a result of exposure to SDs is fated to develop no further than a bract. That such leaf primordia are not immutable bract primordia was clearly demonstrated, however, in *Chenopodium*; plants exposed to only 2 SDs underwent a typical period of rapid initiation of leaf primordia yet the primordia so formed developed into normal foliage leaves (see Part II). It thus seems that the final size and shape of these leaves is not determined by the rates at which their primordia are initiated.

(*c*) *Initiation of axillary bud primordia.* For the purposes of this discussion, axillary bud primordia will be divided into two types as defined earlier: 'vegetative' bud primordia, and the axillary tissues of double ridges. In vegetative plants of *Chenopodium*, axillary bud primordia typically were absent from the axils of the seven or eight youngest leaf primordia. The onset of SD treatment stimulated the initiation of 'vegetative' bud primordia in the axils of the older leaf primordia. When new leaf primordia were initiated, precocious initiation of bud primordia occurred in their axils giving rise to the formation of double ridges.

Similar precocious formation of axillary bud primordia has also been reported to occur at this stage of development in Biloxi soybean (Borthwick and Parker, 1938), *Lobelia dortmanna* (Philipson, 1948), *Trifolium subterraneum* (Aitken, 1955), *Reseda lutea* (Bersillon, 1958) and *Kalanchoë blossfeldiana*

(Stein and Stein, 1960). It must therefore be regarded as a very widespread phenomenon in relation to the transition of the apex to a reproductive state.

Observations that precocious formation of axillary bud primordia almost invariably leads to their eventual development into reproductive tissues have led to the concept that such primordia are fundamentally different in nature from the primordia of 'vegetative' axillary buds. It now seems probable, however, in the light of the following evidence, that the two types of lateral meristem are homologous:

1. In the present investigation, SD treatment stimulated the initiation of 'vegetative' bud primordia in the same way as the axillary tissues of double ridges.

2. The capacity of precocious axillary bud primordia to develop vegetatively under abnormal conditions is frequently apparent. When the short-day plant *Kalanchoë blossfeldiana* is exposed to minimal SD treatment, the precocious bud primordia so induced develop vegetatively (Harder, 1948). Similarly, in *Chenopodium*, several of the double ridges initiated on the 2-SD plants grew through a vegetative phase before becoming entirely reproductive

3. Reeve (1943) and Philipson (1949) have presented fairly strong anatomical evidence which shows the time and position of cell divisions leading to the initiation of the two types of bud primordia to be similar.

On the basis of the present results, therefore, and contrary to the hypothesis proposed by Grégoire (1938) suggesting the existence of a basic difference between a vegetative and floral apex, it seems likely that the transition to flowering does not lead to a sudden irreversible morphogenetic change. The processes stimulating apical growth are not characteristic of reproductive organs alone. The precocious formation of axillary bud primordia does not necessarily lead to flower initiation, and even when leaf primordia are initiated rapidly they are capable of developing into either bracts or foliage leaves.

The evidence available points to the general conclusion that the transition of a plant from the vegetative to the reproductive condition is accompanied by a pronounced stimulation of cell division at the apex which affects all aspects of apical organogenesis, and that this stimulation is somehow directly related to the onset of flowering. There are two main possibilities with regard to the nature of this relationship. These are: (*a*) that internal stimuli leading to the initiation of double ridges simultaneously influence processes which bring about an acceleration of growth and (*b*) that SD treatment induces a stimulation of cell division which in turn leads to the initiation of double ridges.

On the basis of results with gibberellic acid (GA), the second possibility (*b*) seems the more likely. GA almost invariably induces bolting in long-day plants and usually promotes their flowering in non-inductive conditions. There are several reported instances in which plants have bolted but not flowered in response to GA (e.g. Lona, 1959), but the reverse effect—flowering without bolting—has not been observed. Such a situation could be expected to occur occasionally on the basis of the first possibility but is more readily explained by the second. Reasoning of this type is probably also valid for short-day plants. Although there is little evidence that application of GA can bring about the initiation of double ridges in such species in non-flowering conditions, GA has been shown to induce flowering in *Pharbitis nil* (Ogawa and Imamura, 1958). In this species, as in long-day plants, such treatment also leads to an increase in the rate of initiation of leaf primordia (Okuda, 1959).

Investigations by the Snows (1942), using surgical techniques, have shown that in *Epilobium* and *Stachys* the growth of axillary bud primordia near the stem apex is probably inhibited by the apical meristem. It is thus possible that inductive conditions remove a general growth inhibition throughout the apex and thereby allow rapid initiation of leaf primordia and uninhibited development of bud primordia. In this case it might be that the development of an axillary bud initial into reproductive rather than vegetative tissue is a direct consequence of its precocious uninhibited growth. In other words, the release of meristematic tissues from a regulatory inhibition which is present during the vegetative state might lead to the promotion of morphogenetic processes which culminate in the initiation of double ridges. The biochemical nature of such a regulatory inhibition within the apical cells awaits investigation.

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Measurements of the Velocity of Translocation

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With ten Figures in the Text

ABSTRACT

The progress of radioactive sucrose translocated in willow bark was followed by assay of frequent samples of sieve-tube exudate from a severed aphid stylet. The time profile of the advancing radioactive sucrose was the same shape as, and coincident with, the distance profile of radioactivity extracted from a series of sections of the bark. From the slopes of comparable parts of the two curves estimates of the velocity of movement of the distance profile through the bark were obtained and these were checked by independent measurements of a different kind. All velocities measured were about 2 cm.hr.$^{-1}$ Sugar from more than one sieve-tube must be flowing out of the stylet. The logarithmic relation between radioactivity and distance is shown to be only an approximation to a much more complicated relation.

INTRODUCTION

THE theoretical background to these experiments, together with an account of the relevant literature, has been published as a review (Canny, 1960*a*), and to this the reader is referred for a necessary introduction to the work. In that review it was shown: (1) that the customary method of measurement of rate of translocation by radio-tracers is invalidated by the published reports showing translocated radio-tracer to be so distributed in its advance in the plant body that the logarithm of the radioactivity is a linear function of distance; (2) that the slope of the line: log(radioactivity)—distance was a matter for further experimental investigation and the precise location of the profile in the tissues a matter of considerable importance; (3) as a consequence of this logarithmic profile, measurements of radioactivity made at a point on the plant as the radioactive front passes by should show that log(radioactivity) was linearly related to time; (4) that the general proposition that the velocity of any moving profile

$$v = \frac{dx}{dt} = \frac{\partial r/\partial t}{\partial r/\partial x},$$

where $\partial r/\partial t$ and $\partial r/\partial x$ are measured at the same point, may be applied to the particular case of the profile of radioactivity as in equation (5) of that paper to give

$$v = \frac{l}{b}\cdot\frac{d}{dt}\,(-\log r), \tag{1}$$

where r is the radioactivity (in arbitrary units), and b is the slope of the line

Annals of Botany, N.S. Vol. 25, No. 98, 1961.]

log(r)—distance. We use $\frac{d \log r}{dt}$ and $\frac{d \log r}{dx}$, because on published evidence these should be constants. If the distance is measured in cm., the units of b are cm.$^{-1}$; and if time is measured in hours, the velocity is in cm.hr.$^{-1}$ In practical terms, if we measure the rate of change of radioactivity with time at a place on a plant organ carrying radioactive tracer, and then dissect and extract the organ and estimate the fall of radioactivity with distance, the former slope divided by the latter gives us the velocity of advance of the radioactive profile.

The experiments to be described show the application of this method in measuring the velocity of translocation of sucrose in willow stems. The radioactivity of the sucrose in the phloem at different times is conveniently determined by establishing the flow of sieve-tube exudate out of an aphid stylet (Mittler, 1958), and determining the radioactivity of the exuded sucrose solution at intervals. The distribution of radioactive sugar with distance in the phloem is easily determined by cutting up the bark, extracting the sucrose, and assaying it for radioactivity. The status of sieve-tube exudates and their relation to the translocation stream is uncertain, but several of the uncertainties will be cleared up in the present paper, and the validity of the aphid-stylet exudate for this purpose will be shown.

METHODS

Cuttings of three- to four-year-old wood of *Salix viminalis*, *S. alba*, and *S. fragilis* were grown in pots, and cultures of the aphid *Tuberolachnus salignus* maintained on them. Cuttings used in the experiments had leafy side shoots 10 to 20 cm. in length, and aphids feeding on the main stem in the vertical plane of the side shoot. The aphid stylet was severed and the exuded sap collected in calibrated micropipettes of capacity about 1 μl. The volume of each pipette corresponding to measured lengths of filling was known and the volume of each sample collected was measured by the length of pipette it occupied. Samples of sap were commonly collected for a quarter of an hour, in which time 0·25 to 0·5 μl. of sap was exuded. A second pipette was then substituted for a further quarter of an hour. The measured sap sample was spotted on an inch-wide strip of acid-washed Whatman No. 1 chromatographic paper. The radioactivity of the spot could be assayed before or after chromatography with a Geiger tube type EW3H. The more diffuse spot produced by chromatography gave a lower count-rate, and, as the radioactivity present was almost wholly in one substance, sucrose, it was permissible to take advantage of the higher count-rate obtained from the spot before running.

Chromatograms were developed in ethyl acetate/pyridine/water (80:20:10) for 22 hours and sugar spots revealed by autoradiography and the dip of Zimmerman (1957). The sucrose spots were eluted (Canny, 1960*b*), hydrolysed for 20 minutes in 0·01 N. HCl at 100° C., and determined by the method of Wager (1954).

Radioactive carbon was introduced into the plant by feeding about 10–20 μc. of $^{14}CO_2$ (liberated from $BaC^{14}O_3$ by lactic acid) to a single leaf of the side shoot in the daylight of a greenhouse or illuminated by a tungsten-filament microscope lamp. The quantity of $Ba^{14}CO_3$ supplied was recorded, but because of incomplete fixation by the leaf and leaks in the chamber surrounding the leaf, this represented only the maximum radioactivity in the application leaf. The apex and expanding leaves were removed from the application shoot to encourage translocation towards the main stem.

After the radioactive front had passed the exuding stylet a transverse razor cut was made in the bark at the position of the stylet, a strip of bark about 1 cm. wide was removed from the stem in the vertical line up to the point of insertion of the side shoot from which the radiocarbon had come, and down the stem to a point 10–20 cm. below the stylet. These strips were divided into segments 0·5 cm. long and each segment extracted in 80 per cent. ethanol. The ethanolic extracts were evaporated, plated, and assayed for radioactivity to give the distribution of labelled sucrose in the bark. This extraction was shown to remove 95 per cent. of the radiocarbon in the bark by wet-ashing both the residual bark and the extract and counting the two samples as barium carbonate. (For details of this method see Canny and Markus, 1960.)

Precision of the Measurements

Radioactivity was assayed to an accuracy of ±1 per cent. (standard error); sample time to one part in 450. The error in volume measurement varied with the pipette from 0·01 μl. to 0·04 μl. The two most inaccurate measurements were those of sugar concentration (±3 μg.) and lengths of bark sections. The latter were cut by hand using a razor blade and steel rule, so the $\frac{1}{2}$-cm. sections might have varied in area by some 5–10 per cent. Since we are using composite measurements—the slopes of curves, the total accuracy will be greater than that of individual readings. The precision of the estimated slopes may be gauged from the experimental curves presented.

RESULTS

The Radioactive Components of the Phloem Sap

Radiographs of the developed chromatograms of small samples of radioactive sap revealed only one radioactive substance, sucrose. With much larger samples of sap and longer exposures to X-ray film, two very much smaller radioactive spots were revealed, one at R_f 0, and one between sucrose and the origin, but much nearer the latter. These two substances contributed so little to the radioactivity of the sap sample that they were neglected and not further investigated. For all practical purposes the assays of radioactivity made by counting the spotted sap on paper before development give measures of the radioactivity of the sucrose.

Time-course of Radioactivity and Other Characteristics of the Exudate

The variations with time of the several quantities measured may be studied in Table 1 which is the record of a single experiment. In this experiment the exuding stylet was above the treated shoot and it is the upward translocation that is being measured. The volume flow of sap and the mass transfer of sucrose are remarkably steady for the period of the experiment, and it matters little whether the radioactivity of the sap is expressed per unit volume, per μg. of sucrose, or per 15-minute period. The precision of measurement of the sucrose is lower than that of time (the sudden fluctuations of sugar content in Table 1 are probably due to errors), so except where there are large systematic fluctuations of flow rate or sugar content, the radioactivity of the sap is expressed as counts per minute per 15-minute period. This quantity is plotted against time from application of $^{14}CO_2$ in Fig. 1. The logarithm of this quantity to the base 10 is given in the last column of Table 1, and plotted against time from application in Fig. 2. The figures carry the experimental data to longer times than the table, and the dotted line represents a sample of sap collected continuously for 7 hours.

TABLE 1

Sucrose Content and Radioactivity of Phloem Exudate

(Distance from application leaf to main stem: 2·6 cm. Distance from insertion of application shoot to exuding stylet: 5·8 cm. upwards.)

Time from application of $^{14}CO_2$, hours	μg. sucrose	Volume μl.	Counts per minute	Flow rate μl./hr.	Mass transfer μg./hr.	Concentration of exudate μg./μl.	Specific activity c./m./μg.	log (c./m./15 min.)
0	12	0·19	0	0·76	48	63	0	—
0·25	14	0·41	0	1·64	56	34	0	—
0·5	14	0·37	0	1·48	56	38	0	—
0·75	17	0·45	0	1·80	68	38	0	—
1·0	4	0·45	0	1·80	16	9	0	—
1·25	17	0·37	0	1·48	68	46	0	—
1·5	20	0·40	0	1·60	80	50	0	—
1·75	21	0·45	0	1·80	84	47	0	—
2·0	13	0·44	0·18	1·76	52	30	0·014	$\bar{1}$·25
2·25	24	0·45	1·0	1·80	96	53	0·067	0·02
2·5	22	0·40	2·9	1·60	88	55	0·13	0·46
2·75	20	0·38	7·4	1·52	80	53	0·37	0·87
3·0	25	0·42	12·5	1·68	100	60	0·50	1·10
3·25	25	0·34	17·1	1·36	100	74	0·68	1·23
3·5	19	0·35	20·3	1·40	76	54	1·08	1·31
3·75	6	0·42	28·5	1·68	24	14	4·75	1·45
4·0	20	0·39	33·6	1·56	80	51	1·68	1·53
4·25	22	0·40	32·4	1·60	88	55	1·47	1·51
4·5	24	0·39	26·2	1·56	96	62	1·10	1·42
4·75	27	0·42	26·4	1·68	108	65	0·98	1·42
5·0	22	0·36	26·8	1·44	88	61	1·22	1·43
5·25	20	0·39	26·2	1·56	80	51	1·31	1·42
5·5	20	0·39	29·1	1·56	80	51	1·45	1·46

No radioactivity appears in the sap for about 2 hours. Once detected the radioactivity increases ever more rapidly for a period; then its rate of increase falls off; it reaches a maximum, falls slightly, and remains steady. The slopes of the curve, the height of its peak and its position on the time axis depend on the conditions of the experiment, but the form is always of the kind figured.

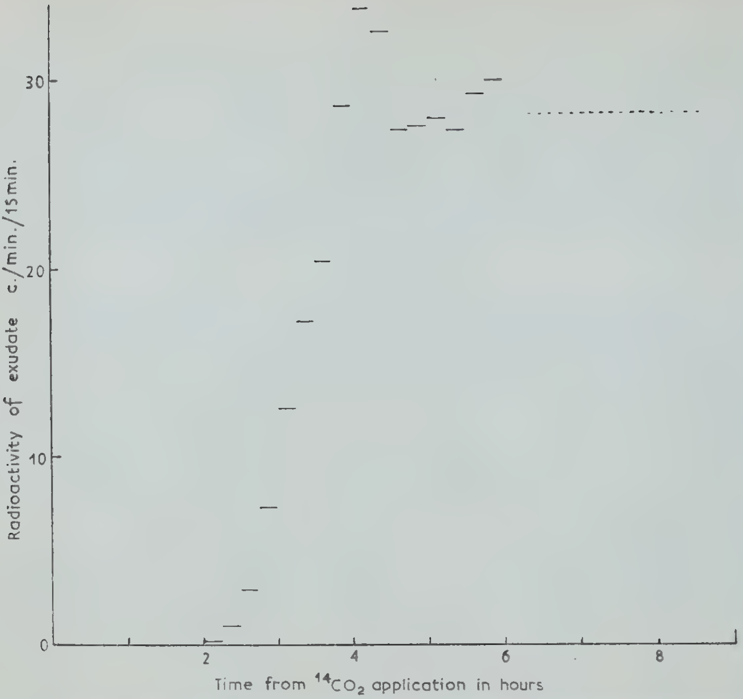

FIG. 1. Radioactivity of 15-minute samples of stylet exudate at intervals after the application of $^{14}CO_2$ to a leaf. The dotted line represents a sample collected continuously for $7\frac{3}{4}$ hours.

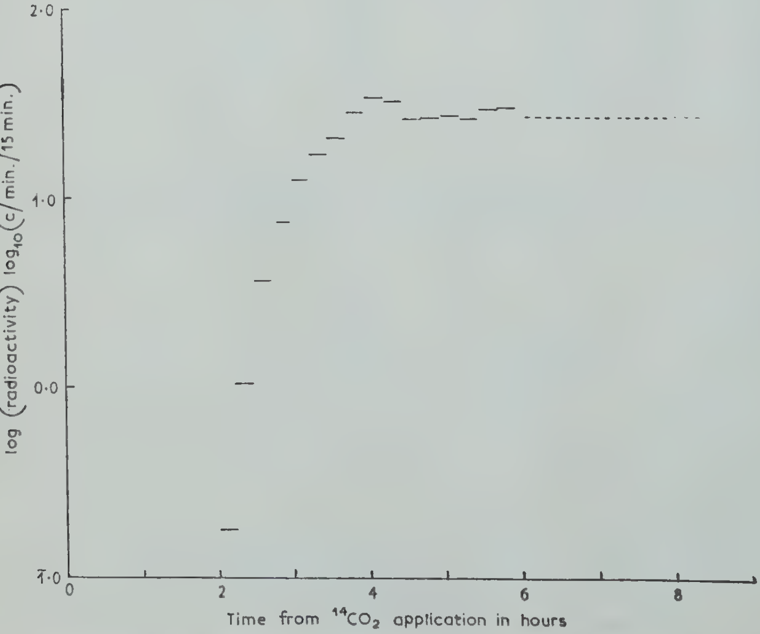

FIG. 2. The data of Fig. 1 replotted with the radioactivity scale logarithmic. The rise of radioactivity with time is only approximately logarithmic.

From Fig. 2 it will be seen that the rise, lasting about 2 hours, is not strictly logarithmic though approximately so for the 1st hour. It could perhaps be regarded as two logarithmic lines of different slopes. The precise shape does not matter; we can still use it to measure the velocity if we know the distribution of radioactivity with distance in the stem.

The Distribution of Radioactivity with Distance in the Stem

No detailed investigation has been made of the chemical compounds containing the ^{14}C in the bark, but all existing evidence suggests that careful extraction would reveal only sucrose-^{14}C, at any rate during the first hours. The extracts made of radioactive translocate from vine petioles (Canny, 1960*c*) and bracken (Hamilton and Canny, 1960) are strictly comparable to the bark extracts made here, and, even if the willow is different, the point does not affect the conclusions presented.

Just as the radioactivity-time relation was found to be not strictly logarithmic in these experiments, so the radioactivity-distance relation showed the same sort of deviations. The profile of radioactivity may be first observed in the side shoot from 1 to 3 hours after application of $^{14}CO_2$ to a leaf. It is then a profile in which the logarithm of the radioactivity is a linear function of distance, and the slope of the line varies with the time at which the extracts were made. After 4 to 5 hours the front is advancing in the bark of the main stem, both upwards and downwards from the point of insertion of the application shoot, with slightly different forms in the two directions. Most experiments have so far been concentrated on the downward-moving front, and the following description applies to that.

The advancing profile is shown in Fig. 3 on linear axes and in Fig. 4 with the radioactivity scale logarithmic. To avoid confusion with the activity-time diagrams the latter have been plotted with a positive slope for the front of radioactivity, while the activity-distance diagrams have a negative slope. The resemblance to the radioactivity-time curves is apparent in the two constant features of those curves: the small maximum before the steady value is reached, and the sinuous front which may be regarded as two logarithmic lines of different slopes. In addition, near the limits of detection of the advancing radioactivity, the distance curve bends forward and the slope is less than that of the main rise. This feature is revealed in the log(radioactivity)-time curves only when a high dose of $^{14}CO_2$ is given. Presumably this part of the time curves is below the limits of detection of the Geiger tube in many experiments. These similarities lead us to expect that the samples of phloem sap obtained from the aphid stylet are indeed samples from a profile of the form of Figs. 3 and 4 moving through the plant past the stylet. Further strong confirmation of this is obtained when both are measured simultaneously.

Simultaneous Measurement of the Distance and Time Curves

Fig. 5 displays the rise of radioactivity with time in the exuded sap up till the sample next after the peak. At this time the cut was made in the bark at the position of the stylet and the bark extracts made to give the plot of radioactivity against distance shown in Fig. 6. The position 'O' on the distance axis is the site of the stylet, and a comparison of the two curves will show that the peak of radioactivity in the bark has just passed the stylet when the radioactivity of the exuded sap began to fall. This result has a number of very important implications which may be summed up by saying that it shows the exuded sap from a single stylet is a good sample of the broad front moving in a large band of sieve-tubes in the bark.

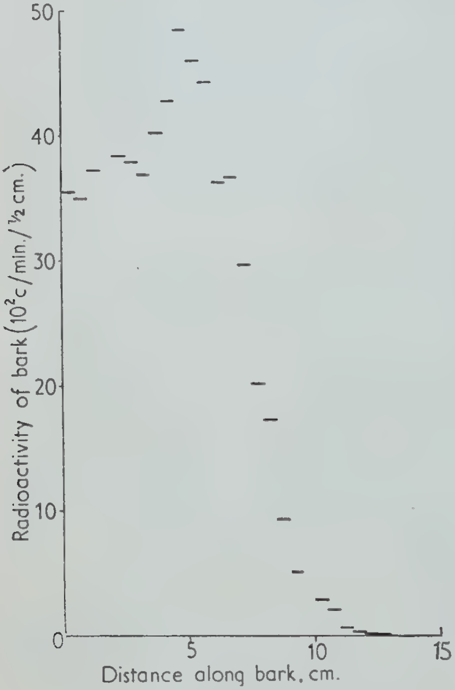

FIG. 3

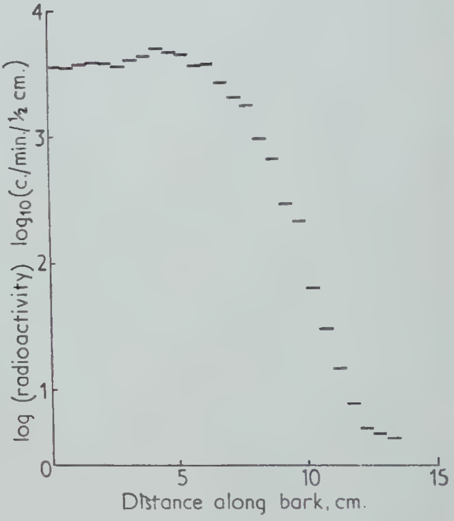

FIG. 4

FIG. 3. The distribution of radioactivity in the bark with distance away from the application shoot. The similarities of shape to the curve in Fig. 1 are apparent.

FIG. 4. Data of Fig. 3 with the radioactivity scale logarithmic. Compare with Fig. 2.

Without this evidence at least three serious objections could be raised to the method here employed, namely: (1) that the aphid stylet is inserted into a sieve-tube vacuole and is withdrawing the vacuolar sap, whereas the translocation may be going on in the cytoplasm, wall, or even in other cells, and the equilibration between the sap and the moving substances might be slow; (2) damage to the phloem functions may be caused by the aphid's injecting saliva, or by the act of puncturing (see Kloft, 1960); (3) the flow from the aphid stylet may be abnormal, either faster or slower than the gross movement of translocated substance. That none of these objections constitutes a serious obstacle is shown by the coincidence of the peaks of radioactivity.

There must be rapid equilibration between the moving sugar and the sugar exuded from the stylet. Moreover, the position of a given radioactive concentration must be the same in nearly all cells carrying labelled sucrose, otherwise the sharp peak of the time profile would be obscured in the bark extracts. There can be very little scatter or variation between the advancing fronts in different cells of the phloem.

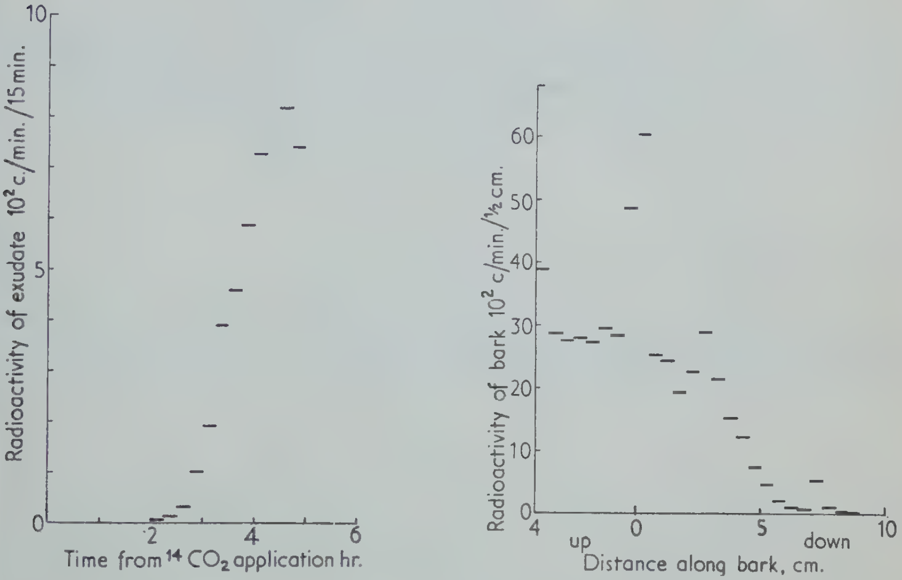

FIG. 5

FIG. 6

FIGS. 5 and 6. Simultaneous measurement of time and distance profiles of radioactivity. At the time when the peak of the time profile has just passed by, the peak of the distance curve is found in the bark just beyond the aphid. The two minor peaks on the rise of Fig. 6 are caused by intermittent illumination of the application leaf through a faulty electrical connexion. The fluctuations have little effect on the slope of the profiles.

There is a further important point to be noticed about the relative sensitivities of the two measurements. Since we know that the peaks on the two curves correspond to each other we can find other points on the curves that correspond. For example, the secondary peak at half maximum peak height in Fig. 6 may well correspond to the point near 400 c./min./15 min. in Fig. 5 which is slightly higher than the smooth curve suggested by the other points. Using this criterion we see that the sensitivity of detection of the radioactivity in the $\frac{1}{2}$-cm. sections of bark is slightly greater than that in the 15-minute samples of sap, but the two are sufficiently close to make the possibility of confusion small. This enables us to say that a particular part of the time curve was produced by a particular part of the profile passing the stylet, and hence to get estimates of the velocity of propagation of the profile from equation (1) even though the experimental curves do not exactly fit the logarithmic relation.

Determinations of Velocities

Returning to equation (1), which assumes that the logarithmic relation holds in both the distance and time curves, it will be noticed that while b is the slope constant of the distance curve and has the units cm.$^{-1}$, the composite

$$\frac{d}{dt}(-\log r)$$

is the slope constant of the time curve and has the units hr.$^{-1}$ It will be necessary to stop referring to b as merely the 'slope constant', and distinguish it as the 'distance-slope constant', while we call 'the time-slope constant'

$$z = \frac{d}{dt}(-\log r).$$

Then the velocity

$$v = \frac{z}{b}. \tag{2}$$

Now while this is true over the whole length of the curves only if the logarithmic relations hold throughout and we have shown experimentally that both depart from the strict relation, the proposition

$$v = \frac{\partial r/\partial t}{\partial r/\partial x}$$

is generally true, and equation (2) holds at corresponding points on the two curves. We may still use the formula over those parts of the curves where there are straight lines on the log r—time and log r—distance plots and where we can be sure, by the criterion just discussed, that the two straight lines selected correspond to the same parts of the front.

Thus in Figs. 7 and 8 the data of Figs. 5 and 6 are plotted with the radioactivity scale logarithmic; the two lines are regarded as derived from the same parts of the front. The slope of the line in Fig. 7 gives $z = 1{\cdot}9$ hr.$^{-1}$ and that of the line in Fig. 8 gives $b = 0{\cdot}65$ cm.$^{-1}$, and the velocity will be $z/b = 2{\cdot}9$ cm.hr.$^{-1}$ The irregular front in this experiment makes the selection of lines more difficult than in the more usual pair shown in Figs. 9 and 10. Here can be seen, in addition, that the result is independent of the part of the curve selected so long as corresponding regions are compared. The pair of lines marked A have $z = 1{\cdot}83$ hr.$^{-1}$ and $b = 0{\cdot}80$ cm.$^{-1}$, giving $v = 2{\cdot}3$ cm.hr.$^{-1}$; and the more dubious pair B have $z = 0{\cdot}31$ hr.$^{-1}$ and $b = 0{\cdot}25$ cm.$^{-1}$, giving $v = 1{\cdot}2$ cm.hr.$^{-1}$ Considering the errors of drawing the lines it would be pressing the method too far to attach any meaning to the decimals of a centimetre, but we may say with confidence that these profiles are moving at $2 \pm 0{\cdot}5$ cm.hr.$^{-1}$ If it is generally true that the same velocity is derived from different parts of the curves then the distance profile is not changing its shape as it moves.

These results are summarized in Table 2 along with additional data for z, b and the derived velocity from other experiments. All the slope comparisons

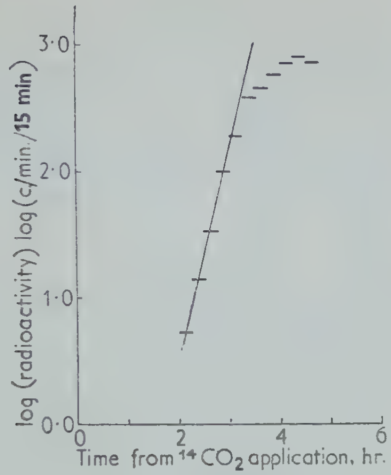

FIG. 7

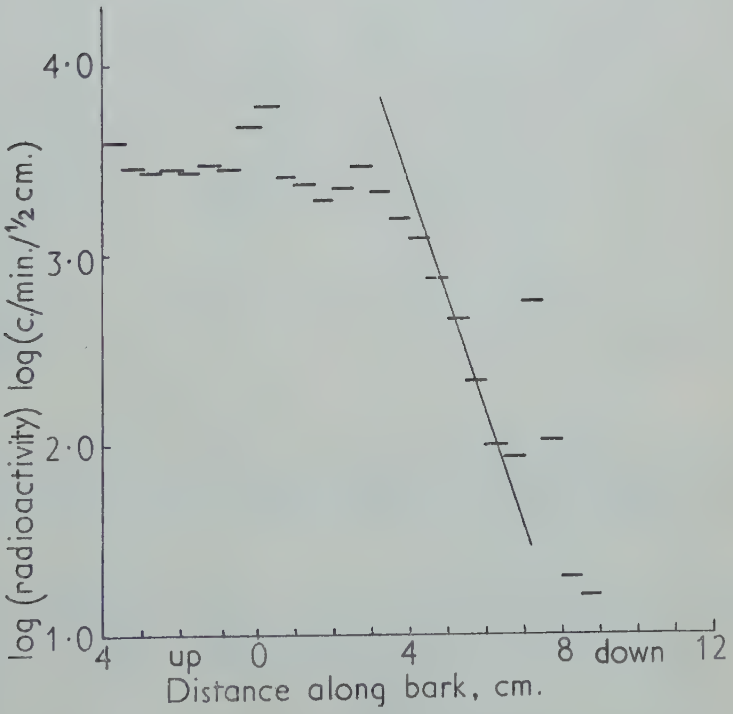

FIG. 8

FIGS. 7 and 8. Data of Figs. 5 and 6 with logarithmic radioactivity scale. The lines were selected as being the slopes of comparable parts of the curves (see text). The slope of the line in Fig. 7 is $z = 1 \cdot 9$ hr.$^{-1}$, and that of the line in Fig. 8 gives $b = 0 \cdot 65$ cm.$^{-1}$ The velocity of the profile, $z/b = 2 \cdot 9$ cm.hr.$^{-1}$

TABLE 2

Measured Velocities of Movement of the Profile

		Measurement from slopes			Measurement from peak position		
Date	Species	Time slope constant z hr.$^{-1}$	Distance slope constant b cm.$^{-1}$	Velocity z/b cm.hr.$^{-1}$	Distance of stylet from application leaf, cm.	Time for peak to reach stylet, hr.	Velocity cm.hr.$^{-1}$
13.7.60	*S. viminalis*	1·90	0·65	2·9	17·7	4·3	4·1
22.7.60	*S. viminalis*	0·51	0·33	1·5	14·4	5·3	2·7
26.9.60	*S. fragilis*	1·83	0·80	2·3	17·5	7·0	2·5
3.10.60	*S. viminalis*	1·69	0·85	2·0	7·8	3·8	2·1
14.4.60*	*S. alba*	1·95	—	—	7·4	4·0	1·9

* Data from Table 1. The bark extracts were not made.

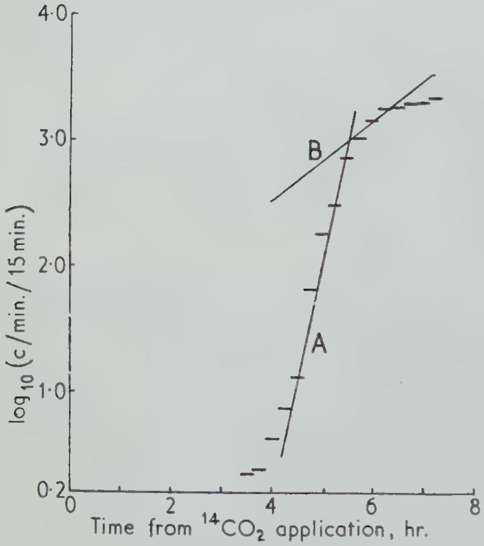

FIG. 9

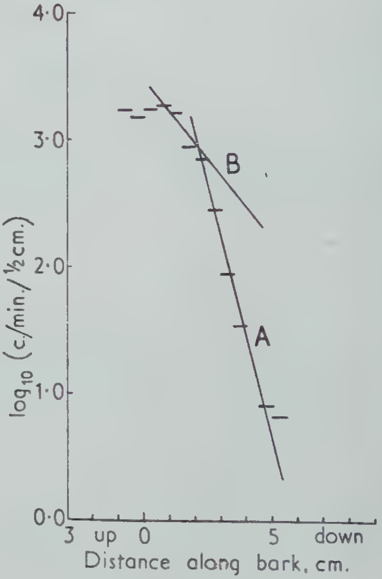

FIG. 10

FIGS. 9 and 10. Further data on the simultaneous measurement of both time and distance profiles. Two values of z can be obtained from Fig. 9 from the two regions A and B. The corresponding regions A and B of Fig. 10 give two values for b. The ratio z/b for either region A or region B gives a value for the velocity. For values see text.

in the table have been made on the main part of the rise like the lines A in Figs. 9 and 10, except in the data of 22.7.60 where the translocated radioactivity was too weak to be detected in the early part of its passage. Similarities in the values of the two slope constants between different experiments will be noticed.

As we have found a peak or marker on the moving radioactive front, we may use the movement of this to supply corroborative measurements of velocity in a more mundane way, dividing the distance the peak has travelled by the time taken. The necessary data are given in Table 2, namely the distance of the exuding stylet from the application leaf and the time from the application of $^{14}CO_2$ at which the peak reached the stylet. The average velocities calculated in this way agree with those instantaneous ones derived in the more sophisticated way from the slopes. A similar check can be made by allowing translocation to continue after the peak has passed the stylet for an hour or two. Then the distance from the peak to the stylet divided by the elapsed time from the passage of the peak gives a third independent measurement of velocity. All the evidence points to velocities around 2 cm.hr.$^{-1}$

DISCUSSION

The Constancy of the Source Radioactivity

The shape of the forward edge of the advancing distance profile could be a consequence of the rate of penetration of tracer into the plant or its fixation, &c., as was emphasized in the antecedent review (Canny, 1960*a*), and the discussion of the method here applied assumed that the radioactivity at the source of translocate should be constant with time. This matter has not been dealt with in detail in this paper but the following considerations show that the success of the method seems to be independent of the radioactivity at the application leaf. With widely varying doses of $^{14}CO_2$, different specific activities, and different presentation times and illuminations, the slopes z and b of the main rise have varied very little. The presentation time to $^{14}CO_2$ is a small fraction of the total rise-time of the profile. The presentation times were generally of the order of $\frac{1}{4}$ to $\frac{1}{2}$ hour, and have been as low as 4 minutes, while the rise-time of the time profile is 2–4 hours. Chance fluctuations in the rate of formation of labelled carbohydrate at the source, like those in the experiment of Figs. 5 and 6, do not lead to drastic changes in the overall slope of the rise. The shape of the advancing profile would thus appear to be a property of the transport system, and not a consequence of events in the application leaf.

Logarithmic Profile and Translocation Profile

The concept that I derived from the published work of a straight-line relation between log(radioactivity) and distance for tracer translocated in the plant, a straight line, moreover, whose slope was constant with different tracers and in different plants, is seen to need revision in the light of more detailed experiments. Over small distances of the plant body the logarithmic relation is valid on the advancing front, but the complete front is something much more complex. The first detectable part of the front is a profile of shallow slope whose value is not precisely determined in the above experiments for it depends on a few determinations near the limits of sensitivity. From curves like that in Fig. 4 we may estimate the slope of this part to be very near that value of 0·1 cm.$^{-1}$ which I derived as an average for b from the published results of experiments lasting up to 30 minutes (Canny, 1960*a*). It seems likely that these experiments were concerned with this part of the front.

Next to arrive is a profile of much steeper slope having a value for b of about 0·7 cm.$^{-1}$ Then as the peak approaches, the slope becomes shallower again, the value of b being around 0·3 or lower. Thereafter the logarithmic slope has no meaning.

The further we investigate the foot of the advancing profile by increasing the sensitivity of detection or the dose of radioactivity, the further forward it seems to curl. I venture to predict that beyond the region of slope $b = 0{\cdot}1$ cm.$^{-1}$ will be found a region of even slighter slope. The two modes of translocation characterized by different 'velocities' found by Nelson, Perkins, and Gorham (1959) may be readily understood as the passing of two regions of this foot of the profile.

The present experiments establish just that location of the position of the tracer profile which the final sentences of my review called in question: 'If the logarithmic profile of radioactivity is found to be established outside the transport system by leakage or accumulation into neighbouring cells, the study of this profile will probably not lead us directly to new knowledge of the transport process.' Previous experiments on the profile had involved no finer dissection of the stem than cutting into short lengths. The exudate from an aphid stylet, on the other hand, comes certainly via the vacuole of a single sieve-tube. The fact that the profile occurs in the sieve-tubes, added to the coincidence of this micro-front with the gross movement in the whole phloem, argues that the profile is a characteristic of the translocation process, not a side reaction of the kind modelled by Horwitz (1958). There may be leakage of translocated sucrose from sieve-tubes into neighbouring cells of the phloem, or even into other tissues, but what leaks is probably a small proportion of what is carried through. Certainly the profile is established independently of side reactions; there is no need to invoke leakage to explain it. It is likely then, that this characteristic profile is a fundamental symptom whose further study promises rewarding information.

Having shown that the profile at the front of the advancing tracer is not logarithmic, we may no longer go on calling it the 'logarithmic profile' as I have done throughout this paper and the review. I shall in future refer to it simply as the 'translocation profile', stating where necessary that it is to be considered either as the double-linear or the semi-logarithmic graph. The two kinds of measurement detailed in this paper will give rise to 'translocation-time profiles' or 'translocation-distance profiles'.

The Measured Velocities

The velocity of movement of the translocation profile has turned out to be surprisingly low. We have been accustomed to think for many years of the velocity of translocation as about 100 cm.hr.$^{-1}$, and during the last few years claims have been made that it is much faster (cf. Nelson *et al.*, 1959). But I have tried to show that the earlier tracer experiments were measuring the wrong thing, and that the later ones were measuring the same wrong thing more sensitively. We must consider what relation the velocity of the translocation profile bears to other measurements of velocity.

The concept of a velocity of translocation arose, as I have pointed out, from the assumption of a mechanism of translocation involving flow of a hypothetical solution. If we try to define it without this assumption we must either define it as the rate of propagation of a disturbance among the moving molecules of sugar (radioactive sugar or a wave of concentration change), or as some kind of average of the velocities of individual sugar molecules. The former seems the only practical possibility. What relation the velocity so defined bears to the velocity of a hypothetical solution, if such exists, is a matter for further experiment to determine, depending on the exchange of material between the hypothetical solution and the surrounding tissues. The situation is analogous to that in the wave theory of light, where the wave velocity is not measurable, only the group velocity of a disturbance imposed on the moving wave.

The rate of advance of the profile may be only the rate of filling up of some space into which radioactive sugar is exchanging, but if this is so, the form of the profile is an extraordinary one, and the laws governing the exchange must be complicated indeed. Further, we have seen that the radioactive sugar in the sieve-tube vacuole is in equilibrium with any radioactive sugar that is outside the sieve-tube vacuole, so this vacuolar space is part of the space into which the radioactive sugar would be exchanging. It is not the primary path of a fast, constant hypothetical flow.

Until further evidence necessitates a retreat to some more complicated hypothesis it will be assumed that the velocity of the translocation profile as defined in the review, and as measured in this paper, does indeed represent the 'velocity of translocation' and does not conceal some more fundamental process of flow characterized by a different velocity. Certainly it is the only useful rate that can be estimated from the advance of tracer molecules.

The only velocity measurements with which my own are comparable are those based on the propagation of a wave of high sugar concentration down the bark of trees and which I discussed at length under the heading 'Chemical Measurements of Velocity' in the review. The velocity of this wave was 2–4 m.hr.$^{-1}$ or about 100 times the velocity measured in this paper, but the comparison need not disturb us since the one set of measurements was made on a full-grown red oak at midsummer, and the other on small rooted cuttings of willow in the laboratory. It seems likely that the velocity of the translocation profile can vary from nearly zero to say 400 cm.hr.$^{-1}$ in different plant organs and under different conditions. In particular, it must be at least ten or twenty times the values measured here in those stem systems where the specific mass transfer was 20 g. dry wt./cm.^{2} sieve-tube/hr., though it need be little different from 2 cm.hr.$^{-1}$ in the petiole systems of Table 1 of the review, where the specific mass transfer of dry weight is only about 3 g./cm.^{2} sieve-tube/hr. We may expect that the velocity will vary from part to part of the same plant.

Mass Transfer of Sucrose

There is another point on which the review must now be corrected in the light of experiment. I showed there that the published values for mass transfer of dry weight through the phloem, reduced to the common unit of g. dry wt./cm.^{2} sieve-tube/hr., all gave values around 20 for stem phloem, and that the data of Weatherley, Peel, and Hill (1959) were apparently in close agreement, giving a value of 24 g. sucrose/cm.^{2} sieve-tube/hr. for the rate of exudation of sucrose out of an aphid stylet in willow stem. No velocity measurements were available for the willow data, but we may use the data of this paper to show that it is not possible that all this sucrose is coming from the cross-section of one sieve-tube, and that the mass transfer figures for sucrose in the willow cutting are probably not near those of the stem systems quoted.

Thus in Table 1 a round figure for the mass transfer of sucrose out of the stylet is 80 μg/hr. This may be arriving at the stylet from both up and down the stem; suppose for the moment that it arrives from one direction only. Assuming the same cross-section for a sieve-tube as Weatherley *et al.*, namely 414 μ^2, and the approximate velocity of 2 cm.hr.$^{-1}$ for the movement of the front, this 80 μg. of sucrose must be contained in a cylinder 20,000 μ long and 414 μ^2 in area, that is of volume $8{\cdot}28 \times 10^6\ \mu^3 = 8 \times 10^{-6}$ cm.^{3} This corresponds to 8×10^{-5} g. per 8×10^{-6} cm.^{3} or 10 g. per cm.^{3} Or, if the sucrose comes from two directions, 5 g. per cm.^{3} Obviously the cross-section across which the sugar is arriving is greater than that of one sieve-tube and the agreement in specific mass transfer figures between the whole plant analyses and the data on stylet exudates is fortuitous. The velocities of the translocation profile in the stem systems where the specific mass transfer was 20 g. dry wt./cm.^{2} sieve-tube/hr. must be much greater than the 2 cm.hr.$^{-1}$ measured on the willow cuttings.

We are forced to the conclusion that the flow of sugar from the stylet is too great to be coming lengthwise in a single sieve-tube, even from both directions. The stylet must be tapping sucrose that is arriving across an area of several, perhaps many sieve-tubes, and work is at present concentrated on finding what this cross-section is.

SUMMARY

1. $^{14}CO_2$ was supplied to leaves of rooted willow cuttings and as the labelled assimilate passed through the bark the exudate was collected from the severed stylet of an aphid that had been feeding on the phloem. The volume, sugar content, and radioactivity of the sap were determined at 15-minute intervals and the shape of the radioactive time profile was shown to be characteristic.

2. The distance profile of radioactivity in the bark was determined by cutting the bark into $\frac{1}{2}$-cm. lengths and extracting and assaying the radioactivity. This profile was shown to have the same characteristic shape as the time profile. The coincidence of fixed points on the two shows that the time profile is produced by the distance profile moving past the exuding stylet, and

that the exudate is a good sample of what is moving in a broad band of phloem near the stylet.

3. From the ratio of the slopes of comparable parts of the two profiles an estimate of the velocity of movement of the distance profile may be obtained. This may be checked by more than one independent estimate of the velocity. All estimates give velocities of about 2 cm.hr.$^{-1}$

4. The shapes of the profiles do not depend on the rate of assimilation of $^{14}CO_2$ by the leaf, and appear to be characteristic of the translocation process.

5. These experiments locate the 'logarithmic profile' found by other workers in stems more precisely in the phloem and sieve-tubes, and at the same time show that the profiles are logarithmic only over limited ranges.

6. The quantity of sucrose that comes from the exuding stylet cannot be arriving across the cross-section of a single sieve-tube at the measured velocity, so the aphid stylet must be drawing on sugar from neighbouring cells besides the one it penetrates.

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A Nutritional Interpretation of Sclerophylly Based on Differences in the Chemical Composition of Sclerophyllous and Mesophytic Leaves

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With three Figures in the Text

ABSTRACT

On the assumption that some of the characteristics of sclerophyllous leaves may have a nutritional basis, data on the leaf composition of nineteen species from evergreen bushland in Jamaica are compared with published data on the leaf composition of thirty-one species of mesophytes from Central America. The data show that in terms of dry matter a sclerophyllous leaf is associated with a decrease in percentage protein content rather than with an increase in percentage fibre content. There is a highly significant positive correlation between the protein and phosphorus contents of the leaves considered, and it is suggested that the ability to tolerate low phosphate may characterize sclerophyllous vegetation in general.

INTRODUCTION

ALTHOUGH the investigations of Maximov (1929), Ashby (1932), and others have placed the study of xeromorphism on a sound scientific basis, particularly by discrediting such teleological concepts as 'physiological drought', many problems of xeromorphism nevertheless remain unsolved. It is true that considerable progress has been made in the understanding of succulence (e.g. Delf, 1912; Pearsall and Ewing, 1929), so that desert and salt-marsh succulents are now recognized as two distinct physiological types, but in the case of sclerophylly the problem of why the same specialized type of leaf should occur in both dry and wet habitats continues to exercise the ingenuity of botanists.

In 1955–6 an ecological study was made (Loveless and Asprey, 1957) of the predominantly sclerophyllous vegetation of the limestone hills of the south coast of Jamaica. This study prompted an investigation into the chemical composition of sclerophyllous leaves (i.e. leaves with increased cutinization and sclerification) on the assumption that such an approach might provide a clue to the factors underlying sclerophylly. For various reasons, including proximity to the University College of the West Indies, the investigation was confined to the vegetation described as Evergreen bushland (Beard, 1949). Leaf samples were collected exclusively from Port Henderson Hill, the locality that was selected previously for the ecological study of this formation in Jamaica.

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Full details of the floristic composition, structure, and physiognomy of evergreen bushland, and of the habitat conditions at Port Henderson Hill, are given in Loveless and Asprey (1957). Evergreen bushland is essentially a scrub dominated by shrubs and small trees that reach an average height of about 4 m. (13 ft.). In Beard's classification of Tropical American vegetation types (Beard, 1955) evergreen bushland is the penultimate member of the Dry Evergreen formation-series, a fact which indicates that it occurs under severe habitat conditions of more or less permanent drought. At Port Henderson Hill there is only one month in an average year with over 10 cm. (4 in.) of rainfall, and there are four consecutive months each with under 2·5 cm. (1 in.). Under such extreme conditions it is not surprising to find that the distinctive appearance of the community is largely due to the prevalent leaf type of the shrubs and small trees (i.e. microphanerophytes). Although the forty-three species of microphanerophytes recorded by quadrat analysis at Port Henderson Hill belong to diverse genera and families, some of the species resemble one another so closely in their leaf characteristics that they are difficult to distinguish except when in flower and fruit. Summaries of the leaf characteristics, based on number of species and frequency, of the microphanerophytes at Port Henderson Hill are given in Table 1. The predominant leaf type is simple, microphyllous, leathery, and evergreen, although mesophytic leaves (belonging to deciduous species) are well represented.

TABLE 1

Summary of the Leaf Characteristics of the Forty-Three Species of Microphanerophytes Recorded in the Evergreen Bushland at Port Henderson Hill, Jamaica

(Data of Loveless and Asprey 1957, based on twenty 5×5 m. random quadrats)

		Raunkiaer's leaf classes				Leaf texture				
	% Simple leaves	% Leptophyll	% Nanophyll	% Microphyll	% Mesophyll	% Leathery	% Mesophytic	% Tomentose	% Hispid	% Evergreen leaves
By number of species	**74·4**	2·3	7·3	**74·4**	16·3	**53·6**	32·6	11·6	2·3	**60·5**
By frequency	**79·5**	0·3	10·6	**76·5**	12·6	**47·1**	35·8	16·1	1·0	**67·2**

In this paper the leaf composition of microphanerophytes from evergreen bushland is compared with that of mesophytes from Central America, and an interpretation of sclerophylly is suggested that may apply to sclerophyllous leaves in general.

MATERIALS AND METHODS

In deciding to make leaf analyses of species from evergreen bushland, it was realized that corresponding results from the leaves of mesophytic plants would be needed for purposes of comparison. Extensive data on the composition of the mesophytic leaves of herbage vegetables from Central America were available in a series of eight papers by Munsell, Williams, Guild *et al.* (1949–50). Because these authors required from their analyses information on the nutritive value of the plants, they determined the following thirteen constituents: moisture, ether extract, crude fibre, nitrogen, ash, calcium, phosphorus, iron, carotene, thiamine, riboflavin, niacin, and ascorbic acid. For the purpose of comparing the composition of sclerophyllous and mesophytic leaves, the author considered that only the moisture, crude fibre, nitrogen, ether extract, ash, calcium, and phosphorus contents were likely to be relevant, and he has therefore confined his attention to these seven constituents. Broadly speaking these seven constituents cover the protoplasmic and skeletal components of the leaf. No account has been taken of reserve materials which presumably form most of the balance between the total composition and the percentage covered by the present analyses.

Collection of Leaf Samples

All the author's samples were collected from plants growing in the field. Healthy mature leaf blades were cut off above the petiole and placed immediately in tightly stoppered, previously weighed bottles. So that the samples should approximate the average composition of the species, each sample included leaves from at least three or four different plants. Within 2 hours of collection the samples were weighed in the laboratory and then oven-dried immediately afterwards. After drying, the samples were reweighed, ground to a powder in a small rotary mill, and then stored for subsequent analysis in brown stoppered bottles kept in the dark.

To cover the possibility of seasonal variation in leaf composition samples were collected in November 1956, March 1957, and July 1957, i.e. at 4-monthly intervals occurring respectively after the main October rains, at the height of the main dry season, and during the minor dry season following the May rains. No definite seasonal trends in composition, such as occur in grass leaves, were observed and the figures given for each species, with the exception of *Bursera simaruba*, are the means of the three separate samples. In the case of *B. simaruba*, which is an obligately deciduous species, no trees were in leaf when the March 1957 samples were taken. For this species, therefore, the figures are the means of the November 1956 and July 1957 samples.

Methods of Chemical Analysis

Except for determining nitrogen and phosphorus, the author has followed the same methods of analysis as those used by Munsell *et al.* (1949–50). Moisture, crude fibre, ether extract, ash, and calcium were all determined by methods of the Association of Official Agricultural Chemists of the United States of America (1945). In the case of nitrogen, whereas Munsell *et al.* adopted the method described by Hamilton and Simpson (1946), the writer used the Kjeldahl method with a selenium/sulphuric acid mixture in the digestion. To facilitate subsequent discussion the nitrogen content of the samples is expressed as percentage crude protein by multiplying the nitrogen figures by 6·25. For the determination of total phosphorus, whereas Munsell *et al.* used a modification of the procedure of Lowry and Lopez (1946), the writer used the following wet digestion method. The ground leaf material was wet-digested with a triacid mixture of sulphuric, nitric, and perchloric acids, and the phosphorus determined by the ammonium molybdate/stannous chloride method using a photo-electric colorimeter for reading the resultant molybdenum blue colour.

Species Selected for Analysis

Since it was not possible for practical reasons to analyse leaves from all the microphanerophytes occurring in evergreen bushland, nineteen species (i.e. just under half the total number) were selected. The data from these nineteen species, together with the results of Munsell *et al.*, would make available data for a total of fifty species. The evergreen-bushland species were chosen so as to cover the range of leaf types encountered in the community and to include representatives belonging to as many different families as possible. The nineteen species, and their leaf characteristics, are given in Table 2. Of the four criteria used in Table 2 for defining leaf type the first two (i.e. form of lamina and leaf size) are self-explanatory. To express leaf

TABLE 2

Leaf Data of the Nineteen Species of Microphanerophytes Selected for Analysis

Species	Family	Form of lamina	Raunkiaer size class	Texture	Seasonality
Bursera simaruba Sarg.	Burseraceae	Cmp	Micro-	Mesophytic	D
Cassia emarginata L.	Caesalpiniaceae	Cmp	Micro-	Mesophytic	D
Piscidia piscipula Sarg.	Papilionatae	Cmp	Micro-	Mesophytic	D
Cordia sebestena Jacq.	Boraginaceae	S	Meso-	Hispid	E
Bourreria baccata Raf.	Boraginaceae	S	Micro-	Tomentose	E
Comocladia velutina Britton	Anacardiaceae	Cmp	Micro-	Tomentose	D?
Lasiocroton macrophyllus Griseb.	Euphorbiaceae	S	Meso-	Tomentose	D?
Croton linearis Jacq.	Euphorbiaceae	S	Nano-	Leathery	E
Capparis ferruginea L.	Capparidaceae	S	Micro-	Leathery	E
Diospyros tetrasperma Sw.	Ebenaceae	S	Micro-	Leathery	E
Sarcomphalus laurinus Griseb.	Rhamnaceae	S	Micro-	Leathery	E
Schaefferia frutescens Jacq.	Celastraceae	S	Micro-	Leathery	E
Gymnanthes lucida Sw.	Euphorbiaceae	S	Micro-	Leathery?	E
Rhus metopium L.	Anacardiaceae	Cmp	Micro-	Leathery	D
Tabebuia riparia (Raf.) Sandwith	Bignoniaceae	Cmp	Micro-	Leathery	D?
Hypelate trifoliata Sw.	Sapindaceae	Cmp	Micro-	Leathery	D?
Pithecellobium unguis-cati Benth.	Mimosaceae	Cmp	Micro-	Leathery	E
Amyris elemifera L.	Rutaceae	Cmp	Micro-	Leathery	E
Guaiacum officinale L.	Zygophyllaceae	Cmp	Micro-	Leathery	E

KEY TO SYMBOLS

Form of lamina
S = simple
Cmp = compound

Leaf-size classes
Nano- = nanophyll
Micro- = microphyll
Meso- = mesophyll

Seasonality
E = evergreen
D = deciduous

texture four broad categories were decided upon and the foliage of each species was allocated to the category with which it showed most affinity. These four categories, with a typical example of each, are as follows: mesophytic (e.g. *Bursera simaruba*), hispid (e.g. *Cordia sebestena*), tomentose (e.g. *Comocladia velutina*), and leathery (e.g. *Guaiacum officinale*). Although the leaves of most of the nineteen species could be classified readily on this basis, some are transitional (e.g. *Gymnanthes lucida* between leathery and mesophytic) while others combine the characteristics of two categories (e.g. hairiness and leatheriness in *Bourreria baccata*). The arbitrary nature of classifying leaves according to texture will be discussed later. The classification of species in the tropics as either evergreen or deciduous is also often arbitrary. Different definitions have been proposed as to what should be regarded as evergreen and deciduous species, and doubtless each has its advantages from a particular point of view. It is probably simplest 'to regard as evergreen those in which the tree bears a substantial number of leaves at all times of the year; deciduous species are those which become bare, or almost so, if only for a few days' (Richards, 1952, p. 195). This usage is followed here. The difficulty remains, however, that some species vary in their seasonal behaviour and would be classified on the above basis as deciduous in one year and evergreen in another. The seasonal behaviour assigned to each species in Table 2 represents what is considered its normal behaviour in an average year at Port Henderson Hill.

RESULTS

Munsell *et al.* give a detailed description of the appearance of each of their samples, from which it is possible to decide the botanical nature of the material analysed. Some of the samples classified as herbage vegetables consisted of young shoots or even entire plants. These have been rejected for the present purpose. Samples from thirty-one species consisted of leaves only and the results from the analysis of these species are shown in Table 3. In their original papers Munsell *et al.* express all values per 100 g. of the raw edible material, but in Table 3 these values (apart from moisture content) have been recalculated on a dry-weight basis. Only the values for the seven constituents determined in the 'sclerophyllous' leaves from Port Henderson Hill are included. The number of samples for each species is also recorded. The corresponding results for the nineteen 'sclerophyllous' species from Port Henderson Hill are given in Table 4.

On the basis of the figures in Tables 3 and 4, the average composition of the 'mesophytic' series of leaves analysed by Munsell *et al.* (1949–50) differs markedly in certain respects from that of the 'sclerophyllous' series analysed by the author. Although the leaves of the herbage vegetables analysed by Munsell *et al.* have so far been described loosely as mesophytic, and those from the evergreen bushland at Port Henderson Hill as sclerophyllous, such a clear-cut distinction is unjustified. So-called sclerophyllous leaves are merely typical of one end in a continuous range of leaf types showing

TABLE 3

Composition of 'Mesophytic' Leaves from Central America

(*Data of Munsell* et al. *1949–50*)

Family	Species	No. of samples	% Moisture content	% Crude fibre (dry-wt. basis)	% Crude protein (dry-wt. basis)	% Ether extract (dry-wt. basis)	% Ash (dry-wt. basis)	% Calcium (dry-wt. basis)	% Phosphorus (dry-wt. basis)
Aizoaceae	*Tetragonia expansa* Thunb.	8	92·1	8·89	33·00	5·05	20·37	0·81	0·44
Amarantaceae	*Amarantus hybridus* L.	3	86·7	13·97	32·19	4·03	19·00	2·16	0·58
Chenopodiaceae	*Beta vulgaris* var. *cicla* Moq.	7	91·6	10·66	32·25	3·78	17·70	0·83	0·49
	Beta vulgaris var. *crassa* Alef.	1	90·6	12·76	29·38	4·26	20·53	0·97	0·36
	Chenopodium ambrosioides L.	1	85·0	8·00	41·69	5·13	16·40	2·28	0·39
	Chenopodium berlandieri Moq.	1	83·0	14·71	42·19	5·47	20·47	0·92	0·40
	Spinacia oleracea L.	2	90·9	9·62	32·82	4·27	23·10	0·59	0·61
Compositae	*Lactuca sativa* var. *capitata* Hort.	3	95·2	11·23	25·68	2·88	10·69	0·40	0·72
	Lactuca sativa var. *intybacea* (Jacq.) Hort.	1	92·4	13·47	20·37	4·74	13·02	0·46	0·43
	Lactuca sativa var. *romana* Hort.	5	94·9	12·32	23·25	4·43	16·35	0·90	0·58
Convolvulaceae	*Ipomoea batatas* (L.) Lam.	2	86·3	11·25	23·00	5·96	9·25	0·81	0·22
Cruciferae	*Brassica campestris* L.	3	89·6	10·09	37·38	6·25	12·70	2·06	0·48
	Brassica juncea var. *foliosa* Bailey	2	90·6	12·31	27·82	3·99	18·22	0·84	0·43
	Brassica oleracea var. *acephala* DC.	1	82·1	7·26	28·69	6·82	10·73	1·67	0·29
	Brassica oleracea var. *capitata* L.	14	91·8	10·14	20·25	2·73	17·75	0·58	0·37
	Brassica pekinensis (Lour.) Rupr.	1	94·2	10·35	27·07	2·59	11·72	0·62	0·70
	Nasturtium officinale R. Br.	4	94·2	11·48	39·38	5·16	19·48	1·72	0·77
Cucurbitaceae	*Cucurbita pepo* L.	2	89·1	15·43	44·07	4·47	15·19	1·06	1·04
	Sechium edule (Jacq.) Sw.	5	89·7	11·84	38·94	4·72	11·20	0·49	0·96
Euphorbiaceae	*Cnidoscolus aconitifolius* (Mill.) I. M. Johnston	2	79·8	10·24	29·63	6·35	9·74	1·16	0·38
	Manihot esculenta Crantz	8	78·5	11·68	29·56	5·30	8·05	0·91	0·43
Labiatae	*Mentha citrata* Ehrhart	1	89·0	8·18	31·13	6·09	15·37	1·60	0·45
Malvaceae	*Malva parviflora* L.	2	81·1	10·46	32·00	3·69	13·62	2·30	0·61
Papilionatae	*Crotalaria longirostrata* Hook. & Arn.	7	82·3	10·44	40·18	4·58	7·83	1·30	0·42
Piperaceae	*Piper auritum* H. B. K.	1	83·8	9·88	22·13	6·61	14·56	1·22	0·33
Portulacaceae	*Calandrinia micrantha* Schlecht.	1	93·0	12·85	30·87	4·57	19·72	0·69	0·44
	Portulaca oleracea L.	5	91·3	10·50	20·93	5·74	17·86	0·85	0·31
	Talinum triangulare (Jacq.) Willd.	1	90·3	8·25	27·45	5·77	23·40	1·24	0·28
Solanaceae	*Solanum nigrum* L.	3	86·4	10·36	36·76	6·08	12·28	1·47	0·44
Umbelliferae	*Eryngium foetidum* L.	1	85·7	13·98	16·38	4·62	14·47	2·06	0·32
	Petroselinum crispum (Mill.) Mansfield	2	84·7	9·48	25·19	4·12	14·35	1·45	0·30
		Mean	88·3	10·45	30·38	4·84	15·32	1·18	0·48

TABLE 4

Composition of 'Sclerophyllous' Leaves from Jamaica

Family	Species	No. of samples	% Moisture content	% Crude fibre (dry-wt. basis)	% Crude protein (dry-wt. basis)	% Ether extract (dry-wt. basis)	% Ash (dry-wt. basis)	% Calcium (dry-wt. basis)	% Phosphorus (dry-wt. basis)
Anacardiaceae	*Comocladia velutina* Britton	3	56·48	8·3	9·63	4·52	4·15	0·99	0·08
	Rhus metopium L.	3	53·25	13·6	9·44	6·95	—	1·20	0·06
Bignoniaceae	*Tabebuia riparia* (Raf.) Sandwith	3	53·30	18·5	8·69	4·16	6·65	1·94	0·07
Boraginaceae	*Bourreria baccata* Raf.	3	50·53	13·2	11·93	3·44	9·88	1·81	0·07
	Cordia sebestena Jacq.	3	55·58	13·4	13·31	5·52	13·15	7·06	0·10
Burseraceae	*Bursera simaruba* Sarg.	2	64·20	9·3	10·38	2·49	—	1·21	0·07
Caesalpiniaceae	*Cassia emarginata* L.	3	59·55	9·8	18·25	6·81	6·18	2·19	0·13
Capparidaceae	*Capparis ferruginea* L.	3	53·00	10·9	18·81	4·57	9·70	3·14	0·07
Celastraceae	*Schaefferia frutescens* Jacq.	3	47·60	21·6	13·25	9·77	10·30	1·73	0·06
Ebenaceae	*Diospyros tetrasperma* Sw.	3	50·05	11·8	9·13	5·71	6·10	1·29	0·06
Euphorbiaceae	*Croton linearis* Jacq.	3	47·96	19·1	15·25	4·12	8·15	0·81	0·10
	Gymnanthes lucida Sw.	3	46·78	11·0	10·87	5·72	6·18	2·13	0·16
	Lasiocroton macrophyllus Griseb.	3	49·50	19·9	13·88	6·76	6·58	3·07	0·10
Mimosaceae	*Pithecellobium unguis-cati* Benth.	3	49·03	17·7	17·75	4·04	9·16	4·59	0·14
Papilionatae	*Piscidia piscipula* Sarg.	3	57·05	17·4	13·06	5·52	9·15	2·39	0·07
Rhamnaceae	*Sarcomphalus laurinus* Griseb.	3	48·59	13·4	11·00	0·86	6·42	2·10	0·08
Rutaceae	*Amyris elemifera* L.	3	38·02	16·9	11·50	4·65	7·60	1·99	0·07
Sapindaceae	*Hypelate trifoliata* Sw.	3	50·52	12·0	8·44	2·53	7·45	2·50	0·05
Zygophyllaceae	*Guaiacum officinale* L.	3	42·34	15·6	11·93	3·61	6·54	1·74	0·06
		Mean	51·28	14·4	12·45	4·83	7·86	2·31	0·08

increasing degrees of sclerophylly. There is no sharp discontinuity between sclerophyllous and mesophytic types, so that for many leaves it is difficult, and even meaningless, to classify them as belonging to either one type or the other. This difficulty was encountered by the author in recording the leaf characteristics of the species listed in Table 2. For the purpose of determining how leaf composition changes with increasing sclerophylly, therefore, it was decided to treat the data of Munsell *et al.* and those of the author as one series (i.e. to assume that there is no fundamental difference between them) and then observe any significant trends that accompany increasing sclerophylly.

Criterion of Sclerophylly

The prefix *sclero-* implies by derivation the degree of hardness of a leaf. It might be thought therefore that percentage crude fibre of dry weight would provide a convenient index of sclerophylly. The mean figures (Tables 3 and 4) show, however, that when fibre content is expressed in this way, the difference between the two sets of leaves is not great (i.e. 10·5 per cent. compared with 14·4 per cent.). This seemingly paradoxical result is explained by the fact that dry-weight measurements ignore the absolute amount of dry matter in a leaf, which is about four times greater in the sclerophyllous series of leaves (with an average moisture content of 51·3 per cent. of fresh weight) than in the mesophytic series of leaves (with an average moisture content of 88·3 per cent. of fresh weight). Any estimate of sclerophylly based on fibre should therefore be expressed in terms of some measure of foliar tissue. Two obvious possibilities present themselves, either to express fibre content on a fresh-weight basis or to express it per unit of leaf protoplasm. Because the moisture content of leaves, particularly of mesophytic types, is subject to diurnal variation, estimates of sclerophylly based on fibre fresh weight will vary for a given leaf according to the time of day at which it is sampled. This source of diurnal variation is eliminated if crude protein is used as a measure of leaf protoplasm, and degree of sclerophylly is expressed by the ratio

$$\frac{\text{Crude fibre dry weight} \times 100}{\text{Crude protein dry weight}}.$$

An additional advantage of expressing sclerophylly in this way is that it does not define fibre content in terms of another unit (such as fresh weight) which itself includes fibre. In view of these two considerations the fibre/protein ratio is considered a better estimate of sclerophylly than crude fibre fresh-weight, and will be the standard measure of sclerophylly used in this paper.

Leaf Composition in Relation to Degree of Sclerophylly

In order to relate leaf composition with degree of sclerophylly, the results for the fifty species analysed by Munsell *et al.* and the author are listed in Table 5 in ascending order of their fibre/protein ratios. It is interesting to note that the range of this ratio for the thirty-one 'mesophytic' species (i.e. from 19·19 to 85·35) overlaps with that for the nineteen 'sclerophyllous' species (i.e. 53·70 to 212·89), so that the combined data form a more or less continuous series. This overlap between the two original sets of data supports the method of treating them as one continuous series rather than that of comparing one set with the other.

(i) *Relation of degree of sclerophylly to moisture content.* The figures in Table 5 show clearly a reciprocal relationship between fibre/protein ratio and moisture content. Statistical treatment of the figures gives a correlation coefficient of $-0{\cdot}85$, P being $< 0{\cdot}001$. It is apparent, therefore, that percentage moisture content could be used as an index of sclerophylly but, in view of the diurnal variation in moisture content of leaves, it would almost certainly not be as reliable as one based on dry-weight measurements.

(ii) *Degree of sclerophylly in relation to crude fibre and crude protein contents.* Although the fibre/protein ratio is used in this paper as the standard estimate of sclerophylly, it cannot be used for correlating degree of sclerophylly with either the fibre or the protein fractions separately. As an alternative estimate, therefore, percentage moisture content will be used. Since the leaves under consideration vary in moisture content from under 40 per cent. to over 90 per cent., the effect of errors inherent in the use of moisture content as an estimate of sclerophylly will be small. It has already been mentioned that on a dry-weight basis the average crude fibre content of the 'sclerophyllous' series of leaves is only slightly higher (14·4 per cent. compared with 10·5 per cent.) than the average for the 'mesophytic' series. It is not surprising, therefore, to find that the correlation between moisture content and crude fibre dry weight, although highly significant ($P < 0{\cdot}001$), is not strong ($r = -0{\cdot}53$). There is, by contrast, a greater degree of correlation between moisture content and protein dry weight. Statistical treatment of the relevant data gives a correlation coefficient of $+0{\cdot}78$ ($P < 0{\cdot}001$) and a regression equation for protein dry weight on moisture content of $x = -9{\cdot}15 + 0{\cdot}4408y$. Consideration jointly of the fibre and protein relations forces one to the rather unexpected conclusion that in terms of dry matter a sclerophyllous leaf is associated more with a decrease in percentage protein content than with an increase in percentage fibre content (Fig. 1). In using the fibre/protein ratio as an index of sclerophylly, therefore, it is a decrease in the denominator rather than an increase in the numerator that is mainly responsible for increases in the value of this ratio. An effect of low nitrogen in contributing to xeromorphy has been observed by Mothes (1932). Using water cultures of *Nicotiana rustica* L. and other species Mothes showed that nitrogen deficiency led to such features as smaller leaf cells, hence greater stomatal frequency, more sclerified tissue, thicker cuticle, greater osmotic pressure, and lesser ash content as percentage of dry weight (but greater as a percentage of fresh weight), all of which are typical features of sclerophyllous leaves.

(iii) *Relation between degree of sclerophylly and crude ether extract.* The results in Table 5 give no indication of any significant correlation between the fibre/protein ratio and crude ether extract. It was observed at the outset of this investigation that, in microscopic sections of some of the sclerophyllous

Chemical Composition of Leaves as Related to Degree of Sclerophylly

(Species arranged in ascending order of their fibre/protein ratio)

Species	Index of sclerophylly*	% Water content	% Crude fibre (dry-wt. basis)	% Crude protein (dry-wt. basis)	% Ether extract (dry-wt. basis)	% Ash (dry-wt. basis)	% Calcium (dry-wt. basis)	% Phosphorus (dry-wt. basis)	Source of data
1. *Chenopodium ambrosioides*	19·19	85·0	8·00	41·69	5·13	16·40	0·81	0·44	Munsell *et al.*
2. *Brassica oleracea* var. *acephala*	25·31	82·1	7·26	28·69	6·82	10·73	1·67	0·29	"
3. *Crotalaria longirostrata*	25·98	82·3	10·44	40·18	4·58	7·83	1·30	0·42	"
4. *Mentha citrata*	26·28	89·0	8·18	31·13	6·09	15·37	1·60	0·45	"
5. *Tetragonia expansa*	26·94	92·1	8·89	33·00	5·05	20·37	0·81	0·44	"
6. *Brassica campestris*	26·99	89·6	10·09	37·38	6·25	12·70	2·06	0·48	"
7. *Solanum nigrum*	28·18	86·4	10·36	36·76	6·08	12·28	1·47	0·44	"
8. *Nasturtium officinale*	29·15	94·2	11·48	39·38	5·16	19·48	1·72	0·77	"
9. *Spinacia oleracea*	29·31	90·9	9·62	32·82	4·27	23·10	0·59	0·61	"
10. *Talinum triangulare*	30·05	90·3	8·25	27·45	5·77	23·40	1·24	0·28	"
11. *Sechium edule*	30·41	89·7	11·84	38·94	4·72	11·20	0·49	0·96	"
12. *Malva parviflora*	32·69	81·1	10·46	32·00	3·69	13·62	2·30	0·61	"
13. *Beta vulgaris* var. *cicla*	33·05	91·6	10·66	32·25	3·78	17·70	0·83	0·49	"
14. *Cnidoscolus aconitifoliu*	34·56	79·8	10·24	29·63	6·35	9·74	1·16	0·38	"
15. *Chenopodium berlandieri*	34·89	83·0	14·71	42·19	5·47	20·47	0·92	0·40	"
16. *Cucurbita pepo*	35·01	89·1	15·43	44·07	4·47	15·19	1·06	1·04	"
17. *Petroselinum crispum*	37·63	84·7	9·48	25·19	4·12	14·35	1·45	0·30	"
18. *Brassica pekinensis*	38·23	94·2	10·35	27·07	2·59	11·72	0·62	0·70	"
19. *Manihot esculenta*	39·51	78·5	11·68	29·56	5·30	8·05	0·91	0·43	"
20. *Calandrinia micrantha*	41·63	93·0	12·85	30·87	4·57	19·72	0·69	0·44	"
21. *Amarantus hybridus*	43·40	86·7	13·97	32·19	4·03	19·00	2·16	0·58	"
22. *Beta vulgaris* var. *crassa*	43·43	90·6	12·76	29·38	4·26	20·53	0·97	0·36	"
23. *Lactuca sativa* var. *capitata*	43·73	95·2	11·23	25·68	2·88	10·69	0·40	0·72	"
24. *Brassica juncea* var. *foliosa*	44·29	90·6	12·31	27·82	3·99	18·22	0·84	0·43	"
25. *Piper auritum*	44·65	83·8	9·88	22·13	6·61	14·56	1·22	0·33	"
26. *Ipomoea batatas*	47·91	86·3	11·25	23·00	5·96	9·25	0·81	0·22	"
27. *Brassica oleracea* var. *capitata*	50·07	91·8	10·14	20·25	2·73	17·75	0·58	0·37	"
28. *Portulaca oleracea*	50·12	91·3	10·50	20·93	5·74	17·86	0·85	0·31	"
29. *Lactuca sativa* var. *romana*	52·99	94·9	12·32	23·25	4·43	16·35	0·90	0·58	"
30. *Cassia emarginata*	53·70	59·6	9·8	18·25	6·81	6·18	2·19	0·13	Original
31. *Capparis ferruginea*	57·95	53·00	10·9	18·81	4·57	9·70	3·14	0·07	Original
32. *Lactuca sativa* var. *intybacea*	66·13	92·4	13·47	20·37	4·74	13·02	0·46	0·43	Munsell *et al.*
33. *Eryngium foetidum*	85·35	85·7	13·98	16·38	4·62	14·47	2·06	0·32	Munsell *et al.*
34. *Comocladia velutina*	86·19	56·48	8·3	9·63	4·52	4·15	0·99	0·08	Original
35. *Bursera simaruba*	89·60	64·20	9·3	10·38	2·49	—	1·21	0·07	"
36. *Pithecellobium unguis-cati*	99·72	49·03	17·7	17·75	4·04	9·16	4·59	0·14	"
37. *Cordia sebestena*	100·68	55·58	13·4	13·31	5·52	13·15	7·06	0·10	"
38. *Gymnanthes lucida*	101·20	46·78	11·0	10·87	5·72	6·18	2·13	0·16	"
39. *Bourreria baccata*	110·65	50·53	13·2	11·93	3·44	9·88	1·81	0·07	"
40. *Sarcomphalus laurinus*	121·81	48·59	13·4	11·00	0·86	6·42	2·10	0·08	"
41. *Croton linearis*	125·25	47·96	19·1	15·25	4·12	8·15	0·81	0·10	"
42. *Diospyros tetrasperma*	129·24	50·05	11·8	9·13	5·71	6·10	1·29	0·06	"
43. *Guaiacum officinale*	130·76	42·34	15·6	11·93	3·61	6·54	1·74	0·06	"
44. *Piscidia piscipula*	133·23	57·05	17·4	13·06	5·52	9·15	2·39	0·07	"
45. *Hypelate trifoliata*	142·18	50·52	12·0	8·44	2·53	7·45	2·50	0·05	"
46. *Lasiocroton macrophyllus*	143·37	49·50	19·9	13·88	6·76	6·58	3·07	0·10	"
47. *Rhus metopium*	144·07	53·25	13·6	9·44	6·95	—	1·20	0·06	"
48. *Amyris elemifera*	146·96	38·02	16·9	11·50	4·65	7·60	1·99	0·07	"
49. *Schaefferia frutescens*	163·02	47·60	21·6	13·25	9·77	10·30	1·73	0·06	"
50. *Tabebuia riparia*	212·89	53·30	18·5	8·69	4·16	6·65	1·94	0·07	"

* i.e. $\frac{\text{\% Crude fibre (dry wt.)} \times 100}{\text{\% Crude protein (dry wt.)}}$

leaves from evergreen bushland, staining with Sudan III revealed the presence of conspicuous globules of fatty material in the mesophyll cells. On this basis a positive correlation between crude ether extract and degree of sclerophylly was anticipated. A connexion between lipoid content and sclerophylly would help to explain the presence of an abnormally thick cuticle in sclerophyllous leaves, and possibly also the postulated decreased permeability of their mesophyll cells. The author considers that the possibility of a connexion between lipoid content and sclerophylly warrants further investigation, but the present negative results indicate that a more precise method of estimating the lipoid content of leaves than extraction with ether would be necessary to investigate such a possibility.

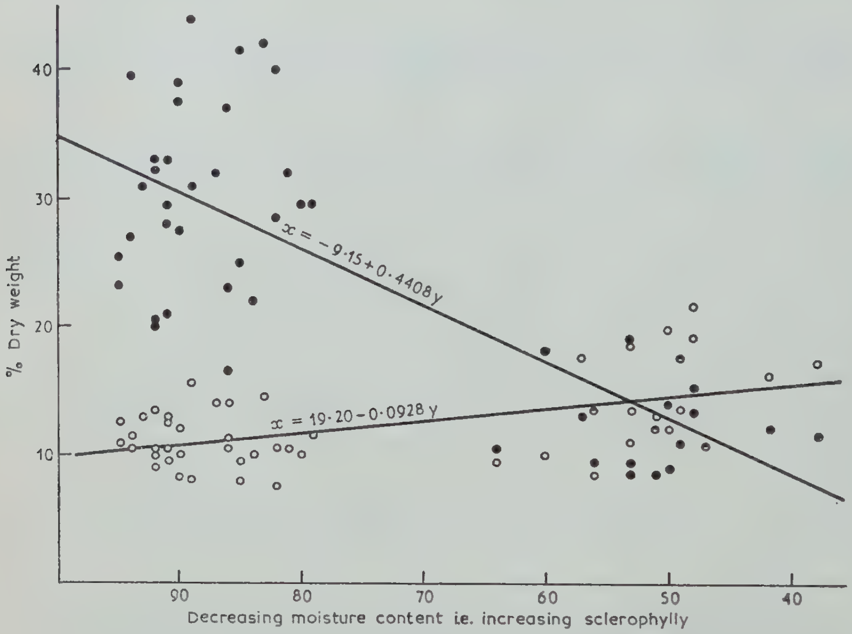

FIG. 1. Regression lines showing that as leaves become more sclerophyllous there is a pronounced decrease in protein content (solid circles) accompanied by only a slight increase in fibre content (open circles). Moisture contents as %

(iv) *Relation between degree of sclerophylly and ash content.* The ash content (Tables 3 and 4) of the 'mesophytic' series of leaves analysed by Munsell *et al.* is on the average double that of the 'sclerophyllous' series analysed by the author. Statistical analysis of the combined data (Table 5) gives a correlation coefficient between the fibre/protein ratio and ash content of $-0{\cdot}61$ ($P < 0{\cdot}001$). Although this result is highly significant statistically it is doubtful, in view of the large number of elements composing the ash fraction and of the varying proportions in which they may occur, if any conclusion can be drawn beyond the suggestion that mineral nutrition may be an important factor in sclerophylly.

(v) *Degree of sclerophylly in relation to calcium and phosphorus contents.* The data for the calcium and phosphorus contents (Table 5) differ markedly from one another in their relation to changes in the fibre/protein ratio. Not only is the general direction of change different (calcium content tends to increase whereas phosphorus content tends to decrease with increase in the fibre/protein ratio), but the pattern of change also differs. There is no significant correlation between calcium content and degree of sclerophylly for either the mesophytic or the sclerophyllous sets of data considered separately, and the correlation ($r = +0{\cdot}33$) for both sets of data combined as one series is significant only at the 1:20 level. In the case of phosphorus content, in spite of the small absolute amount of this element, there is a highly significant overall correlation ($r = -0{\cdot}71$, $P < 0{\cdot}001$) between phosphorus content and the fibre/protein ratio. Nevertheless, when the two sets of data are considered separately, a significant correlation exists only for the sclerophyllous set ($r = -0{\cdot}41$, $P < 0{\cdot}1$ but $> 0{\cdot}05$). The scatter diagram (Fig. 2) relating phosphorus content with the fibre/protein ratio is suggestive of a limiting factor curve, i.e. that the fibre/protein ratio decreases with increased phosphorus content up to a certain level (about 0·3 per cent.) above which increased phosphorus content does not result in a further proportional decrease in the fibre/protein ratio.

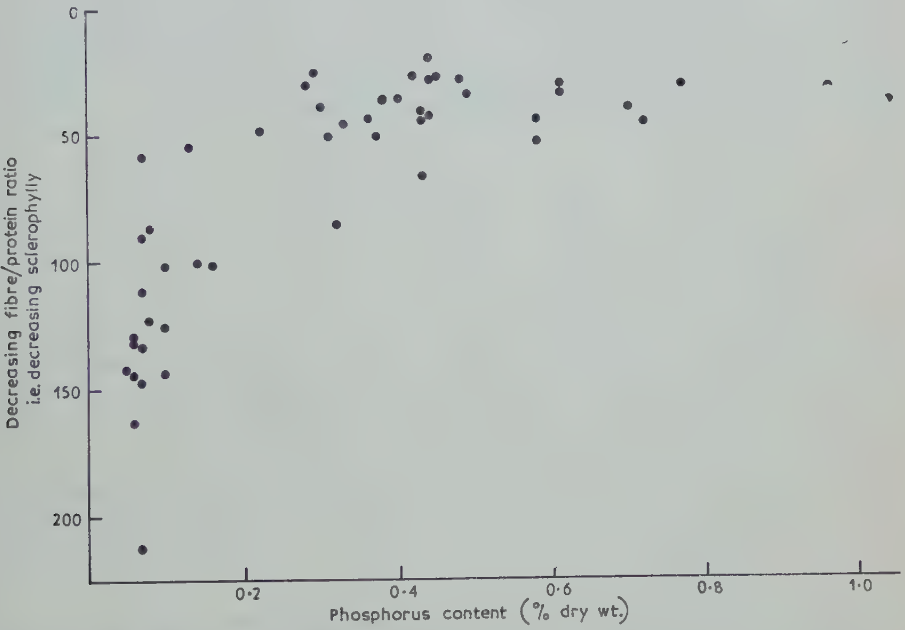

FIG. 2. Leaf phosphorus content in relation to degree of sclerophylly (as measured by fibre/protein ratio).

DISCUSSION AND CONCLUSIONS

One of the basic principles of plant ecology is that 'it is the most severe conditions of habitat, rather than the average, that mainly determine the characteristics of communities' (Salisbury, 1959, p. 126). If the results embodied in this paper are interpreted in the light of this *Principle of Extremes*, it would appear that phosphorus content (which is the fraction showing the greatest relative variation between the low and high ends in the range of sclerophylly) constitutes the factor that is in 'relative minimum' at the sclerophyllous end of the series. In the light of this observation it is reasonable to suggest that phosphate deficiency of the soil might be an important factor in sclerophylly. To support this suggestion the following figures for the analysis of two samples of soil from Port Henderson Hill may be cited.

	pH	Nitrogen %	Available potash (K_2O) p.p.m.	Available phosphate (P_2O_5) p.p.m.
Sample 1	7·8	1·50	636	37
Sample 2	8·1	1·34	530	38

Both samples are alkaline in reaction and have high contents of total nitrogen and available potash. The content of available phosphate is, however, very low, the level of adequacy for most crop plants being considered in the order of 50–60 p.p.m. A combination of such low available phosphate with high total nitrogen is not characteristic of other Jamaican soils.

In view of the broad correlation that exists between vegetation types and particular climatic conditions, it is only natural that the influences of climatic factors in determining the distribution of species and communities should have received the major attention of ecologists. During recent decades, however, the investigations of plant physiologists have emphasized the importance of edaphic factors. Although much is known about the nutrient requirements of crop plants, there are all too few mineral nutrition studies on wild plants, especially those of extreme habitats. Species which can grow on very poor soils must either use minerals extremely efficiently or else have some metabolic compensation which enables them to tolerate these soils. Such physiological adaptations will inevitably have high survival value under the selective influences of extreme types of soil. The importance of the edaphic factor and its strong selective action in the evolution of endemic species and ecotypes has been discussed by Mason and Stout (1954).

The extreme soil factor, apart from dryness, at Port Henderson Hill is phosphate deficiency which, it is suggested, may furnish the clue to the presence of sclerophyllous vegetation in the area. An association of low phosphate with sclerophylly has also been observed by Beadle (1954) in Australia, where phosphate-deficient soils are occupied by sclerophyllous vegetation whereas the adjacent normal soils support rain forest. Laboratory estimation of the relative fertility of the various soils proved that soil nitrogen, though often low, was never the primary limiting factor as commonly believed.

This effect of low phosphate in delimiting sclerophyllous vegetation could be explained if it is assumed that sclerophyllous species can tolerate low levels of this element while most species cannot.

A peculiarity of sclerophyllous vegetation is that it occurs in both dry and wet habitats. The earlier view of Schimper (1903) was that the structure of the sclerophyllous leaf helped to prevent excessive water loss from plants whose root systems were unable to absorb much water, either because of actual physical drought or because of some form of physiological drought. The concept of physiological drought has had to be discarded in the light of experimental evidence, but no alternative explanation has been offered. Although drought cannot explain the presence of the same specialized leaf type in both dry and wet habitats, it is nevertheless reasonable to ask whether there is some factor common to both habitats which might explain the similarity. Phosphate deficiency, either alone or more usually associated with nitrogen deficiency, meets this requirement. Not only is phosphate deficiency characteristic of certain soils that are physically dry, as at Port Henderson Hill, but it 'also characterizes acid soils in areas of high rainfall but especially when these are rich in organic matter since phosphorus in organic combination is even less readily available to the plant than from inorganic compounds such as iron-phosphate' (Salisbury, 1959, p. 139). These conditions (i.e. acid soils in areas of high rainfall) constitute precisely the habitat of moorland and bog vegetation which includes, in Britain, typically sclerophyllous species. In this context it is relevant to mention that Caughey (1945), after an intensive study of the water relations of shrubs characteristic of bogs, suggested that their sclerophyllous appearance might be determined partly by mineral deficiencies.

If, in the light of the above evidence, we accept as a working hypothesis that sclerophylly can be attributed to the effects, particularly on nitrogen metabolism, of phosphate deficiency, then certain features of sclerophyllous leaves assume significance. The point has already been made that, in terms of dry matter, a sclerophyllous leaf is not fibre-rich but protein-deficient. It is now well established that the roles of phosphorus and nitrogen in plant metabolism are interrelated in a number of ways. Phosphorus, like nitrogen, is essential for protein synthesis, and the deficiency symptoms of these two elements are usually very similar. If, in addition, it is a general phenomenon that low phosphate depresses protein synthesis, as has been demonstrated for certain species (e.g. Eckerson, 1931), then the protein deficiency of sclerophyllous leaves can be correlated with their tolerance of low phosphate. The present results (Table 5) support this contention. There is a highly significant overall correlation ($r = +0{\cdot}90$, $P < 0{\cdot}001$) between phosphorus and crude protein (Fig. 3), and this relation also holds for the data of Munsell *et al.* ($r = +0{\cdot}73$, $P < 0{\cdot}001$) and those of the author ($r = +0{\cdot}50$, $P < 0{\cdot}05$) considered separately.

Another feature of sclerophyllous leaves that may be significant in terms of phosphate deficiency is their osmotic pressure relations. Many workers have reported that the leaf sap of xerophytes is more concentrated than that of mesophytes. In general terms, species of mesophytic regions have an average osmotic pressure of about 10 atmospheres whereas species in the Arizona desert have an average osmotic pressure of about 20 atmospheres. Harris and Lawrence (1917), using a cryoscopic method, have shown that the average osmotic pressure of the leaf sap of eleven species of sclerophyllous shrubs (N.B. arborescent species usually have higher osmotic values than herbaceous species) at Port Henderson Hill is 28·6 atmospheres, a value comparable with that of the corresponding growth forms in the Arizona desert. Their figures for species which are included in the present investigation are as follows:

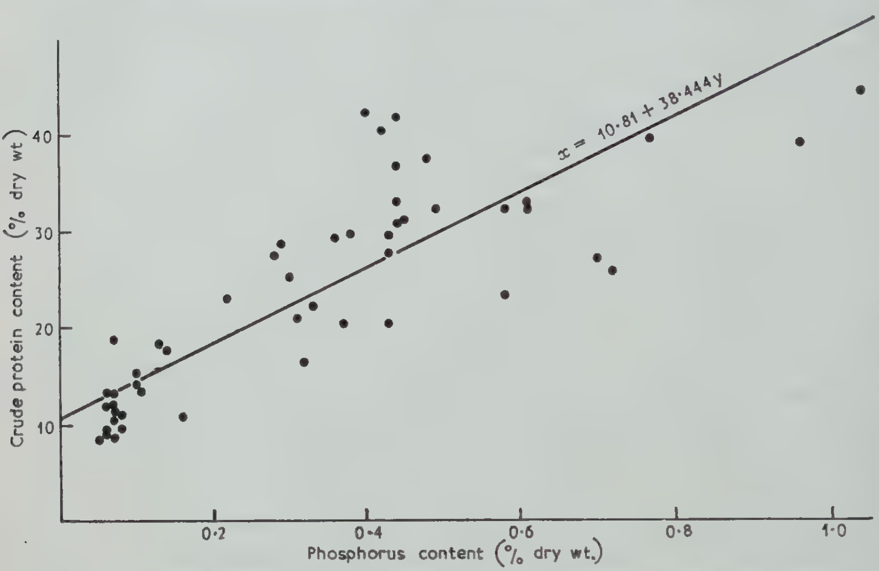

FIG. 3. The regression of crude-protein content on phosphorus content in leaves.

Capparis ferruginea	42·9 atm.
Cassia emarginata	23·6 atm.
Guaiacum officinale	31·9 atm.
Hypelate trifoliata	28·1 atm.
Piscidia piscipula	18·0 atm.
Sarcomphalus laurinus	19·6 atm.

Osmotic values of leaves are affected not only by direct water loss but also by the starch–sugar balance. For a given leaf the value is partly environmentally determined and partly specific in the sense that xerophytes, irrespective of the environment in which they are growing, have characteristically higher osmotic values than mesophytes (Maximov, 1929). Iljin (1929) has shown that the average sugar content of twenty-seven species of xerophytic trees and shrubs (6·89 per cent. of dry weight) was almost twice as much as that of thirty-eight species of mesophytic trees and shrubs (3·6 per cent. of dry weight). The exceptionally high osmotic values of the microphanerophytes at Port Henderson Hill are almost certainly due to high sugar contents. Since phosphate deficiency, like nitrogen deficiency, may be correlated not only with a decrease in protein synthesis but often also with an accumulation of sugar, it is possible that the high osmotic values of sclerophyllous leaves are associated with a metabolism that is geared to a low level of phosphate intake.

In the light of the above considerations it is suggested that phosphate deficiency may play a major part in sclerophylly, but more data are needed before this hypothesis can be accepted. In the past there has been a tendency to interpret the characteristics of sclerophyllous leaves almost entirely in terms of water economy, whereas this paper draws attention to the possibility that many of these characteristics may have a nutritional basis. Such an approach to sclerophylly is complementary to, and in no way conflicts with, the extensive work on drought resistance by Levitt (1956), Iljin (1957), and others.

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Studies on the Germination of Cereals

3. The Effect of the Covering Layers on the Uptake of Water by the Embryo of the Wheat Grain

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With two Figures in the Text

ABSTRACT

The uptake of water by imbibition, vacuolation, and growth of the embryo, has been determined when wheat grains were placed under germination conditions before maturation was completed; and the effect of the covering layers has been investigated in a red and a white variety at three stages during ripening.

The water lost during ripening was replaced by imbibition without causing the embryo to expand sufficiently to rupture the covering layers. The cells of the coleorhiza and epiblast absorbed water by vacuolation as soon as they were imbibed; but a further period elapsed before the scutellum became imbibed and water uptake was associated with an increase in embryo dry weight.

In both varieties, uptake of water by the imbibed embryo was prevented by the inner layer of the pericarp and the testa in unripe grains, and by the epidermal layer of the pericarp during the early stages of ripening. But after the final stage of rapid desiccation the covering layers of the white grains were ruptured when the embryo absorbed water by vacuolation; whereas the covering layers of the red grains remained intact and the uptake of water was delayed until a sufficient period had elapsed for nutrients from the endosperm to be available for growth of the embryo.

The varietal difference in germination is therefore attributed to the greater strength of the covering layers of the red grains, which are not disrupted during ripening, and prevent expansion of the embryo until the water absorbing capacity has been increased by the transfer of reserves from the endosperm.

INTRODUCTION

ALTHOUGH the water relations of seeds are of fundamental importance during ripening and during germination, the relationship between the initial loss of water and the subsequent uptake remains obscure. Many seeds, however, first acquire the ability to germinate during ripening and, in wheat, varietal differences in this respect determine whether the grains are liable to sprout in the ear. In the varieties with white grains the embryos are able to rupture the covering layers when water is available and uptake then proceeds without interruption; but in the varieties with red grains the covering layers remain intact and uptake ceases for a period, depending on the stage of maturation, as soon as the embryos are imbibed. This difference is not due to the pigmentation of the covering layers because both kinds of grains germinate

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readily when they are fully mature; nor is it apparently due to differences in the permeability of the covering layers to water or oxygen (Wellington, 1956*b*). It might, however, be due to a difference in either the pressure exerted by the embryos, or the mechanical resistance of the covering layers; since both would directly affect the critical stage when the covering layers are ruptured.

It has been suggested that the embryos of the red grains cannot overcome the restriction on expansion, imposed by the covering layers, until their capacity for absorbing water has been increased by the transfer of nutrients from the endosperm (Wellington, 1956*b*). The varietal difference in germination behaviour may, therefore, reflect the different extent to which the resistance of the covering layers in the red and the white grains is eliminated during ripening. The purpose of the present investigation has been to follow the uptake of water by the embryos during the successive stages of imbibition, cell vacuolation, and growth; and to compare the effect of the covering layers when the grains of both varieties were allowed to absorb water at different stages during ripening.

EXPERIMENTAL PROCEDURE

The comparison was made between the white grains of *Triticum vulgare* CV. Holdfast and the red grains of *T. vulgare* CV. Atle because the difference in their germination behaviour during ripening was well established (Wellington, 1956*a*). The varieties also had the same anthesis date, when they were sown in the autumn, which ensured that the grains were exposed to identical environmental conditions during ripening. The water absorbed by imbibition was determined by the increase in moisture content of embryos which had been killed before uptake started; and the combined uptake by imbibition and vacuolation of the original cells was determined by the increase in moisture content of live embryos which were excised and placed in water. The total uptake by imbibition, vacuolation, and growth sustained by external nutrients, was determined by the increase in moisture content of live embryos exposed *in situ* on the endosperm or excised and placed in solutions of starch or sucrose. Finally the effect of the covering layers was assessed by the difference between the moisture content of embryos in intact grains and in grains with the embryo exposed.

The substrate for both grains and excised embryos consisted of circles of cellulose wadding in plastic Petri-dishes. At the start the circles were saturated with 20 ml. of distilled water: sufficient to maintain the rate of uptake at a maximum for 120 hours. Each dish was covered with a plastic disc, with a circular hole of 1 cm. diameter in the middle for aeration, and was maintained in an atmosphere of 95–98 per cent. R.H. at a constant temperature of 15° C. The intact grains were placed directly onto the saturated wadding; but those with the embryo exposed first had the pericarp and testa removed from the proximal end with a fine scalpel. The excised embryos were obtained by removing the covering layers from intact grains, which had been kept on the saturated wadding for 4 hours to soften them, and then detaching the embryo with some of the endosperm to prevent damage to the scutellum. After the adhering endosperm had been carefully removed the embryos were transferred to wadding in another dish.

In each treatment replicates of 10 embryos were removed at intervals up to 120 hours and blotted to remove superficial moisture; each embryo was then weighed on a torsion balance, dried for 20 hours at 98° C., and weighed again to determine the dry weight and moisture content. The dry weight and moisture content of the embryos in a random sample of grains was also determined at each time of harvesting.

EXPERIMENTAL RESULTS

Water Uptake by Dead Embryos

The embryos in grains harvested at 16 weeks after anthesis were killed by either heating them at 98° C. for 4 hours or soaking them in a solution containing 2 per cent. mercuric chloride for the same period. Some embryos were then excised, while others were exposed *in situ*, and the remainder were left enclosed by the covering layers; their initial moisture content was 11 per cent. in both varieties and the subsequent increase under germination conditions was determined after both methods of killing. Similar values were obtained in each case so only data relating to the embryos killed by mercuric chloride have been included in Table 1.

TABLE 1

Increase in Moisture Content (% Dry Weight) of Dead Embryos

Time	Red variety			White variety		
(hours)	Excised	Exposed	Enclosed	Excised	Exposed	Enclosed
4	125	113	88	119	96	83
8	154	106	103	163	121	95
16	152	117	122	154	125	127
24	151	130	140	167	140	143
48	175	149	157	190	152	160
72	205	148	151	232	156	167
120	189	155	160	237	159	149

After the first 4 hours the values for the excised embryos were higher than those for the exposed embryos which were in turn higher than those for the embryos enclosed by the covering layers. Although the greater part of the uptake by excised embryos probably occurred during the soaking period, prior to excision, the moisture content was consistently higher when the entire surface of the embryo was free to absorb water than when the scutellum remained in contact with the endosperm. The initial rate of uptake was reduced by the presence of the covering layers in both varieties; but the difference in moisture content between the enclosed and the exposed embryos disappeared at 8 hours in the red variety, and 16 hours in the white, although the covering layers still remained intact. It is therefore evident that they did not limit the total amount of water imbibed by the embryo of either variety.

The moisture content of the excised embryos reached 150 per cent. at 8 hours and then remained more or less constant until 24 hours. The subsequent irregular increases are difficult to explain; they were not due to leaching of material. as there was no reduction in dry weight, but they may have resulted from the greater expansion of the scutellum when detached from the endosperm. The moisture content of the exposed and enclosed embryos increased more slowly, and did not reach 150 per cent. until 48 hours; as there was no further increase this level appears to represent the maximum reached by imbibition alone. This is slightly higher than the moisture content of embryos in unripe grains at 5 weeks after anthesis (Wellington, 1956*a*); but it does indicate that the amount of water absorbed by imbibition merely replaces that lost during ripening.

Water Uptake by Live Embryos

The same treatments were also applied to the embryos in grains which had not been killed and the changes in their moisture content and dry weight during germination are shown in Fig. 1. There was an initial phase of rapid uptake as in the case of the dead embryos; but this was followed by a second phase of rapid uptake after the moisture content had reached the level at which the dead embryos were completely imbibed. The time when the second phase started, and the associated changes in dry weight, depended on whether the embryos had been excised, or were exposed *in situ* or enclosed by the covering layers when uptake began.

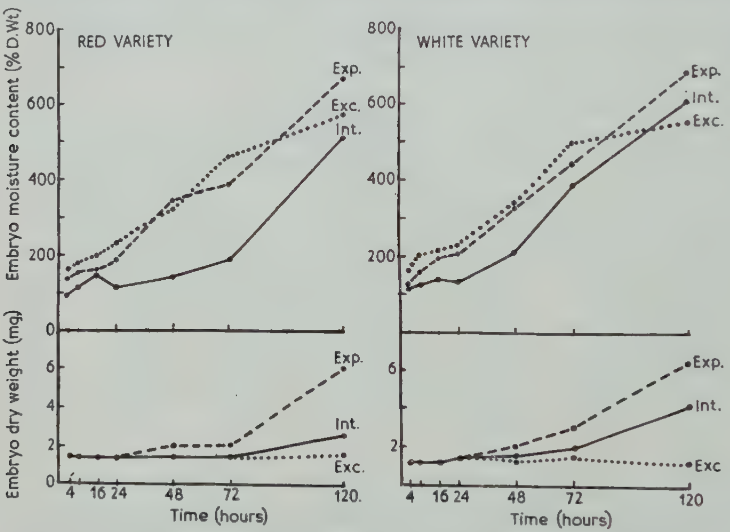

FIG. 1. Changes in moisture content and dry weight during germination of wheat embryos which were excised (Exc.), exposed *in situ* (Exp.), or left in the intact grains (Int.).

The moisture content of the excised embryos already exceeded 150 per cent. at 4 hours and continued to increase at a more or less constant rate until 72 hours; this was followed by uptake at a reduced rate until the end of the test. It therefore appears that the initial phase of imbibition was followed immediately by the second phase of rapid uptake; and as this was not associated with any change in dry weight it must be attributed to vacuolation of the original meristematic tissues of the embryos. This process resulted in a fourfold increase in moisture content between 4 hours and 120 hours but the reduced rate of uptake after 72 hours suggests that it was nearing completion at the end of the test.

The moisture content of the embryos exposed *in situ* was slightly lower than that of the excised embryos at 4 hours, but reached 150 per cent. at 8 hours and 200 per cent. at 24 hours; it then continued to increase at a constant rate, similar to that of the excised embryos between 4 hours and 72 hours, until the end of the test. Since the moisture content of the embryos reached 150 per cent. much earlier when they were alive than when they were dead (Table 1), the second phase of rapid uptake was apparently initiated before imbibition was completed. This may have been due to vacuolation of superficial tissues, such as the coleorhiza and epiblast, before the scutellum was fully imbibed. There was no subsequent reduction in the rate of uptake and the marked increase in dry weight between 72 hours and 120 hours, shown in Fig. 1, indicates that reserve materials from the endosperm were then available for growth. These would be expected to maintain the water absorbing capacity of the embryos by increasing the osmotic pressure of existing cells and by causing new meristematic cells to be formed.

The moisture content of the embryos enclosed by the covering layers also increased rapidly during the first 4 hours. Although the level reached was lower than for either the excised or the exposed embryos, and it did not exceed 150 per cent. until 48 hours in the white variety, and 72 hours in the red, when the covering layers were ruptured and the second rapid phase of uptake began. The rate of uptake was then similar to that of the exposed embryos and there was also an increase in dry weight after 72 hours. This suggests that expansion of the embryos by vacuolation enabled them to exert sufficient pressure to rupture the covering layers in the white variety; because this occurred before the rate of uptake had started to fall in the case of the excised embryos, and before there had been any increase in dry weight in the case of the exposed embryos. But the covering layers in the red variety were apparently strong enough to prevent any expansion of the embryos by vacuolation; and they did not rupture until the reserves from the endosperm were available for growth and greater pressure could be exerted by the embryos as a result of the increase in their water-absorbing capacity.

The effect of the covering layers on vacuolation was confirmed by removing them during uptake by the red grains, at 24 hours and at 48 hours, and determining the increase in embryo moisture content during the next 24 hours. The results in Table 2 show that the increase remained approximately

TABLE 2

Effect of Removing the Covering Layers from Red Grains during Water Uptake

Time (hrs.)	Moisture content of enclosed embryos	Moisture content of embryos after exposure for 24 hrs.	Increased water uptake following removal of covering layers.
0	12	—	—
24	90	134	44
48	108	149	41
72	119	162	43

constant at 40 per cent. although the moisture content of the enclosed embryos increased from 90 per cent. at 24 hours to 119 per cent. at 72 hours. This would be consistent with uptake of water by vacuolation of the cells in the coleorhiza and epiblast, as soon as the embryo was exposed, but before it was completely imbibed.

TABLE 3

Growth of Excised Embryos in Solutions of Starch and Sucrose

	Red variety				White variety			
	Moisture content (% dry wt.)		Dry weight (mg./embryo)		Moisture content (% dry wt.)		Dry weight (mg./embryo)	
Time (Hrs.)	Starch	Sucrose	Starch	Sucrose	Starch	Sucrose	Starch	Sucrose
24	223	205	1·7	1·8	235	214	1·7	1·6
48	339	304	1·8	2·0	333	318	1·9	2·1
72	467	424	1·9	2·5	448	413	2·2	2·3
120	666	472	2·6	3·2	736	589	2·2	3·0

The effect of external nutrients on the water-absorbing capacity of the embryos was demonstrated by placing some which had been excised into solutions containing 2 per cent. soluble starch, or 2 per cent. sucrose, and determining the subsequent increase in their moisture content and dry weight. The embryos of the two varieties behaved in similar ways and the data in Table 3 show that the moisture content of those in the starch solution increased at a rate similar to that of embryos exposed *in situ* (Fig. 1); whereas the moisture content of the embryos in the sucrose solution was consistently lower although their dry weight started to increase at an earlier stage in the test. The total amount of water absorbed by the embryo was the same in each case, but the more rapidly growing embryos in the sucrose had less water per cell and therefore a greater water-absorbing capacity.

In a similar way the availability of nutrients from the endosperm would be expected to increase the water-absorbing capacity of the embryo by growth.

The Effect of the Covering Layers during Ripening

During ripening, three stages can be distinguished by changes in the appearance of the covering layers. The first is the loss of the intense green colour when growth ceases and starch accumulates in the endosperm; the second is the development of the characteristic colour of the variety when pigment is laid down in the testa; and the third is the formation of folds and creases caused by shrinkage of the grain during the final desiccation (Wellington, 1956*b*). In the varieties studied the first stage usually occurs at 5 weeks after anthesis, the second at 7 weeks, and the third at 9 weeks; but the onset of each stage depends on climatic conditions and varies with the season. In the present investigation experiments were made in two season with different climatic conditions which are indicated by the meteorological data in Table 4. The effect of these conditions on the progress of desiccation during ripening is evident from the reduction in moisture content of the embryos which is shown at weekly intervals

TABLE 4

Season Differences in Sunshine, Rainfall, and Embryo Moisture Content during Ripening

	1955				1956			
Weeks after anthesis	Sun (hrs.)	Rain (in.)	Moisture content		Sun (hrs.)	Rain (in.)	Moisture content	
			Red	White			Red	White
4	54	0	195	170	26	0·1	211	178
5	48	0	117	109	12	1·0	163	127
6	46	0·5	95	88	53	0·1	122	115
7	36	1·2	43	48	28	2·2	106	169
8	48	0·2	19	28	31	0·8	40	57
9	39	0·1	24	27	29	1·2	69	78
10	35	0·1	17	13	56	0·3	40	50

There were more hours of sunshine and less rain in 1955 than in 1956 and the moisture content of the embryos was consistently lower at all stages except 4 weeks after anthesis. The seasonal difference was most marked at 7 weeks after anthesis, because the final intense desiccation had started by then in 1955, but was delayed until the 8th week in 1956.

The effect of the covering layers on water uptake by the embryo was investigated at three stages during ripening by allowing grains of both varieties to absorb water under controlled conditions at 5, 7, and 9 weeks after anthesis. The increase in embryo moisture content was then determined in one series with the covering layers intact and in another series with the embryos exposed; a third series was also included in 1956 with the epidermal layer of the pericarp only removed. The changes in moisture content at each stage of ripening are shown separately for the two varieties in Fig. 2.

At 5 weeks after anthesis, the initial moisture content of the embryos exceeded 100 per cent. in both seasons (Table 4) and there was no uptake during the first 24 hours because there had not yet been sufficient desiccation for the imbibitional forces to become effective. In spite of this the moisture content of the embryos exposed *in situ* started to increase rapidly after 48 hours, although there was no increase in the moisture content of the enclosed embryos throughout the test period. In both varieties, therefore, the covering layers prevented the uptake of water and expansion of the embryo associated with vacuolation and growth. This effect was due to the inner layer of the pericarp and the testa because uptake was not promoted when the epidermal layer of the pericarp was removed.

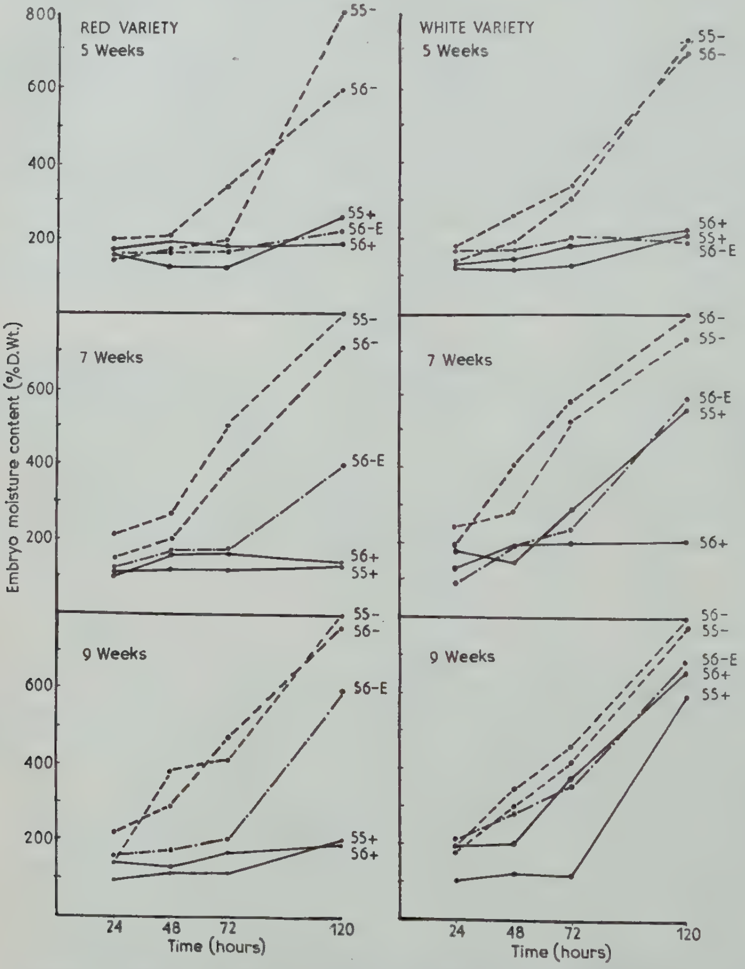

FIG. 2. Changes in moisture content during germination of wheat embryos which were exposed *in situ* (−), or left in intact grains (+), or in grains with epidermal layer of pericarp removed (−E), at three stages of ripening in 1955 and 1956.

At 7 weeks after anthesis, the initial moisture content of the embryos was less than 50 per cent. in 1955, but was still 100 per cent. or more in 1956. The moisture content of the exposed embryos increased rapidly after 48 hours in both seasons; the moisture content of embryos in the intact white grains also started to increase rapidly after 48 hours in 1955, but not in 1956, and there was no increase in the moisture content of embryos in the intact red grains in either season. The covering layers of the white grains had therefore lost their inhibiting effect on water uptake by the embryo after they were subjected to rapid desiccation during ripening in 1955; whereas the covering layers of the red grains were still able to prevent uptake. When the epidermal layer of the pericarp was removed in 1956, however, the moisture content of the embryos started to increase rapidly at 72 hours in both varieties; so at this stage the inhibiting effect was due to this layer and not to the inner layer and the testa.

At 9 weeks after anthesis the initial moisture content had fallen to a level determined by the humidity of the atmosphere; although it was rather higher in 1956 than in 1955, this was due to uptake during rain and not to lack of desiccation, because the moisture content had been lower in the previous week (Table 4). A rapid increase in the moisture content of the exposed embryos was again evident and appeared to start rather earlier than at the previous stage of ripening. There was also a rapid increase in the moisture content of embryos in the intact white grains after 72 hours in 1955, and after 48 hours in 1956, but there was still no increase in the moisture content of embryos in the intact red grains in either season. Removal of the epidermal layer of the pericarp had no further effect on water uptake by embryos in the white grains, except for a slightly higher value at 48 hours, but it again promoted uptake by the embryos in the red grains after 72 hours, as was the case at 7 weeks after anthesis. This layer was, therefore, the main cause of delayed germination which persisted after the red grains were ripe for harvest but was eliminated by the desiccation of the white grains during ripening.

DISCUSSION

When germination of wheat grains is delayed by varietal factors, or lack of maturation, the covering layers remain intact for an extended period and cause a lag in water uptake between the initial phase of imbibition and the subsequent phases of vacuolation and growth. During this lag period a temporary equilibrium appears to exist between the pressure exerted by the expanding embryo and the mechanical resistance of the covering layers. The characteristic germination behaviour, therefore, depends on the relative magnitude of these forces which are determined by the processes involved in water uptake by the embryo and by the changes in the covering layers during ripening of the grain.

During ripening the embryos lose 90 per cent. of their moisture and when the ripe grains are allowed to absorb water this is replaced whether the embryo is alive or dead. The initial uptake is, therefore, due to the physical process of imbibition, and the time required for this stage will depend on the rate of diffusion through the covering layers, which appears to be the same in both varieties (Table 1), and the rate of diffusion from the outer to the inner tissues of the embryo.

When the embryo is imbibed water uptake continues by a process which occurs in the existing cells and is associated with a change from the meristematic to the vacuolated condition. This process does not occur when the embryos are dead, or at low temperature, or in the absence of oxygen, as is evident from the data in Table 5. Although the maximum values were somewhat higher than those reached by the imbibition of the dead embryos (Table 1), the second phase of rapid uptake, which occurs under optimal conditions, was suppressed when live grains with exposed embryos were allowed to absorb water at 5° C., or in an atmosphere of nitrogen. The process is therefore one of active uptake judged by the citeria proposed by Spanner (1952).

TABLE 5

Increase in Moisture Content (% Dry Weight) of Exposed Embryos at 5° C. or in N_2

Time (hrs.)	5° C.		N_2	
	Red	White	Red	White
24	137	151	150	163
48	163	163	115	145
72	—	—	224	187
120	237	211	166	164

When the superficial layers of the embryo become imbibed, during the first 4 hours, the cells become vacuolated and form the typical aerenchyma of the coleorhiza and epiblast. That most of the original tissues of the embryo must also be capable of water uptake by vacuolation is shown by the fourfold increase in moisture content of the excised embryos, after imbibition, without any change in their dry weight.

In the absence of an external source of carbohydrate water uptake by the existing tissues appears to be nearing completion at 120 hours; but when nutrients are available for growth, new meristematic tissues are formed which maintain the rate of uptake. The leading role of the scutellum in mobilizing nutrients from the endosperm has been demonstrated by Edelman, Shibko, and Keys (1959). But the embryo takes longer to complete imbibition when it is in contact with the endosperm than when it is excised (Table 1). It appears that the scutellum may not be completely imbibed until 24–48 hours after uptake starts leading to a corresponding delay before the water-absorbing capacity of the embryo is reinforced by reserves from the endosperm. This delay may also account for the observation of Percival (1921) that degradation of starch in the endosperm first occurred 24 hours after the start of germination.

In the unripe grains the covering layers of both varieties prevent any uptake of water by vacuolation and growth of the embryo. In the early stages of ripening the effect is due to the inner layer of the pericarp and the testa which may either prevent the expansion of the embryo or exclude the oxygen required for these processes. But at a later stage the effect is due to the epidermal layer of the pericarp, and, as additional thickening is laid down on the lateral walls of the cells between 3 and 6 weeks after anthesis (Wellington, 1956*b*), it is probably caused by increased resistance to expansion of the embryo. During the final stage of desiccation the epidermal layer in the white variety becomes separated from the inner layers, and is thrown into a series of folds and creases in the dorsal region by shrinkage of the grain. This would undoubtedly weaken the covering layers and coincides with the loss of their ability to prevent water uptake by vacuolation of the embryo. The covering layers of the red grains apparently have sufficient elasticity to accommodate the final reduction in volume of the grains, without becoming separated or forming folds and creases, and retain sufficient strength to prevent the embryo expanding. But when nutrients eventually become available from the endosperm the embryo is able to exert the additional pressure required to rupture the covering layers in the ripe grains. The importance of these nutrients has been indicated previously by removing the distal end of the red grains so that water was absorbed through the endosperm; this eliminated the lag in water uptake and the covering layers were ruptured by elongation of the coleoptile and seminal roots without any expansion of the coleorhiza (Wellington, 1956*b*). Further evidence is now provided by the increase in moisture content and dry weight of excised embryos when placed in solutions of starch or sucrose.

The increase in water uptake, which resulted when the epidermal layer of the red grains was removed at 9 weeks after anthesis, shows that it still retained sufficient strength to prevent expansion by the embryo when the changes during ripening had been completed. The simplest explanation is that the effect of desiccation under field conditions was not sufficient to disrupt and weaken the covering layers. This would also account for the fact that a greatly increased proportion of red grains were induced to germinate when they had been subjected to further desiccation in atmospheres of low relative humidity after harvest (Wellington, 1956*a*).

SUMMARY

1. A study has been made of water uptake by the embryo during the germination of wheat embryos in order to determine whether the water absorbed during imbibition, vacuolation, or growth, enabled the embryo to exert sufficient pressure to rupture the covering layers. The resistance of the covering layers was also compared in grains of a red and a white variety at three stages during ripening.

2. Uptake by imbibition was more rapid when the embryo was excised than when the scutellum remained in contact with the endosperm. The presence of the covering layers did not affect the total amount of water imbibed but the expansion of the embryo caused by imbibition was not sufficient to rupture them.

3. After they were imbibed the embryos were capable of a fourfold increase in moisture content without any change in dry weight; but the rate of uptake was reduced at the end of the test period. When external nutrients were

available water uptake was maintained at a constant rate and there was an increase in dry weight due to the formation of new tissues.

4. In ripe grains of the white variety the embryo was able to rupture the covering layers when water was absorbed by vacuolation. But the covering layers of the red grains prevented uptake of water by the embryo until a sufficient period had elapsed for nutrients to be available for growth.

5. In unripe grains uptake of water by the embryo was prevented by the inner layer of the pericarp and testa, and during the early stages of ripening by the epidermal layer of the pericarp. The inhibiting effect was lost in the white variety during the final stages of ripening but it persisted in the red variety after the grains were ripe for harvest.

6. It is therefore suggested that the varietal difference in germination behaviour arises because the epidermal layer of the pericarp in the red variety is not weakened by desiccation during ripening to the same extent as in the white.

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Studies on the Germination of Cereals

4. The Oxygen Requirement for Germination of Wheat Grains during Maturation

BY

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ABSTRACT

The oyxgen required by the wheat embryo, for germination at different stages of maturation, has been determined by the number of grains with the embryo exposed which germinated in different oxygen concentrations. Some embryos required only 0·5 per cent., and the majority required less than 5 per cent., but there was also a proportion which required a concentration approaching the level in air. There were more embryos in the last category in the grains of a red variety than in those of a white variety but this did not account for the varietal difference in germination when the grains were intact.

The effect of the covering layers was also determined in each atmosphere by the difference in germination between intact grains and grains with the embryo exposed. The covering layers were sufficiently impermeable to prevent germination in both varieties when the external oxygen concentration was very low; when the concentration was increased from 5 to 60 per cent., however, the inhibiting effect of the covering layers was reduced in the white variety but not in the red. The delayed germination of the red grains was therefore attributed to some factor other than the impermeability of the covering layers to oxygen.

INTRODUCTION

MATURE wheat grains will not germinate when oxygen is completely excluded from the external atmosphere and germination is reduced when the oxygen tension is lower than that in air (Taylor, 1942). Some oxygen must, therefore, diffuse into the grain before the embryo can rupture the covering layers; and it has been suggested that germination of freshly harvested grains may be delayed by the impermeability of their coats (Harrington, 1923). In a previous study (Wellington, 1956) it has been shown that maturation affects the germination of wheat grains in two ways. The first concerns the embryo and is independent of the variety; the second concerns the covering layers and results in a varietal difference in germination as soon as the chlorophyll disappears from the pericarp. When the covering layers have assumed the characteristic colour of the variety, the embryo is able to rupture them in a high percentage of the grains in white varieties, but only in a low percentage of those in red varieties.

The present investigation was undertaken to determine whether the different germination behaviour of the white and red grains could be attributed

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to differences in either the oxygen requirement of the embryos or the permeability of the covering layers. Grains from a red and a white variety were therefore harvested at intervals during ripening and then germinated in atmospheres containing different proportions of oxygen above and below the normal level. The oxygen requirement of the embryos was determined by the number of grains which germinated when the embryos had been exposed; and the effect of the covering layers was obtained from the difference between the germination of intact grains and of grains with the embryo exposed.

EXPERIMENTAL PROCEDURE

Ears of the red wheat *Triticum vulgare* CV. Atle, and the white wheat *Triticum vulgare* CV. Holdfast, were tagged on the first day of anthesis and subsequently harvested at pre-determined intervals, because different stages of maturity could be characterized approximately by the time after anthesis. The covering layers were still green at 5 weeks; the characteristic colour of the grains had developed and marked shrinkage had occurred at 7 weeks; ripening was completed and the moisture content of the grains was determined by the relative humidity of the atmosphere at 9 weeks; and there had been considerable after-ripening although some grains were still not fully mature at 15 weeks.

At each harvesting date the grains were removed from about 20 ears of each variety and were well mixed; they were then divided into two series, one with the embryos completely exposed and the other with the covering layers intact. Replicates from each series were planted in Petri-dishes on cellulose wadding, saturated with 20 ml. of distilled water, and were maintained at 15° C. in the different atmospheres.

Samples of 10 grains were removed after 24, 48, 72, and 120 hours, and the number of embryos with the radicle emerging through the coleorhiza was recorded. This particular stage in germination was a convenient indication that vegetative growth had started and it could be applied to both the exposed and the enclosed embryos. The dry weight and water content of the individual embryos was also determined.

Germination tests were made simultaneously in six different atmospheres by connecting each cylinder, containing a selected gas mixture, to four inverted bell jars sealed to glass sheets with petroleum jelly. The jars accommodated four Petri-dishes on a wire staging containing replicates of the grains with exposed embryos and the intact grains of each variety. Sampling at each interval of the test could, therefore, be done by disconnecting one jar at a time without interrupting the gas supply to the remainder. At the start of the test the gas was introduced at 20 lb. per sq. in., to sweep out the original atmosphere, but after 30 minutes the pressure was reduced to 3 lb. per sq. in. The gas was bubbled through a sloping tube containing distilled water in order to saturate it and to provide a means of comparing the actual rate of flow; it was then introduced at the bottom of each jar and passed over the dishes and out at the top into a second flowmeter to indicate any leakage.

EXPERIMENTAL RESULTS

In the first experiment the ears were harvested at weekly intervals from 4–10 weeks after anthesis, and at 15 weeks after anthesis; they were then germinated in atmospheres containing 5, 10, 30, 40, and 60 per cent. of oxygen, together with air as control. These formed a series with two oxygen concentrations below the normal level for germination and three above it. The results in each atmosphere are shown in Table 1 for the stages of maturation represented by 5, 7, 9, and 15 weeks after anthesis.

TABLE 1

The Effect of Oxygen Concentration, and the Presence of the Covering Layers, on the Germination of Grains at Different Stages of Maturity

Red variety

Oxygen in germination atmosphere	Embryos exposed					Covering layers intact					Effect of covering layers T_2–T_1
	Weeks after anthesis				Total (T_1)	Weeks after anthesis				Total (T_2)	
	5	7	9	15		5	7	9	15		
5%	7	16	15	19	57	0	0	3	2	5	−52
10%	9	22	16	21	68	0	0	1	5	6	−62
Air (control)	11	18	14	24	67	0	2	4	4	10	−57
30%	11	18	13	22	64	0	1	1	8	10	−54
40%	12	18	18	25	73	0	0	4	11	15	−58
60%	12	21	17	23	73	0	2	3	10	15	−58
Total	62	113	93	134	402	0	5	16	40	61	
White variety											
5%	11	25	21	27	84	0	2	8	11	21	−63
10%	8	25	16	27	76	0	4	12	11	27	−49
Air (control)	13	26	17	26	82	0	3	10	17	30	−52
30%	11	23	15	26	75	0	6	7	17	30	−45
40%	11	24	19	26	80	0	7	11	15	33	−47
60%	12	24	19	25	80	0	5	15	17	37	−43
Total	66	147	107	157	477	0	27	63	88	178	

The number of red grains with exposed embryos which germinated in 5 per cent. oxygen was lower than the number in air and the numbers in 40 and 60 per cent. oxygen were higher. But there was no increase in germination with increasing oxygen concentration in the case of the white grains with exposed embryos. The embryos in some of the red grains apparently had a higher oxygen requirement than those in the white grains. In both varieties the numbers of intact grains which germinated formed a gradient with increasing oxygen concentration; the lower results were obtained when the oxygen concentration was less than in air and the higher results when it was greater, although in both cases the differences from the control were small. In the case of the red grains the effect of the covering layers was relatively constant in all the atmospheres because the germination of both the exposed embryos and the intact grains increased with the oxygen concentration. But in the case of the white grains the effect was greatest in 5 per cent. oxygen, and least in 60 per cent. oxygen, which suggests that the permeability of the covering layers to oxygen may account for part of their effect on germination in this variety. The germination of the white grains was, however, consistently higher than that of the red in all atmospheres; and the difference was greatest when the covering layers were intact, except at 5 weeks after anthesis when no germination occurred.

TABLE 2

Ratios of 'F' from the Analysis of Variance of the Water Content of Individual Embryos in Independent Replicates

Treatment	Degrees of freedom	Interval during the test			
		24 hrs.	48 hrs.	72 hrs.	120 hrs.
Oxygen concentration (O)	5	2·09	2·36	1·69	12·3‡
Stage of maturity (M)	7	98‡	52·4‡	131‡	302·7‡
Covering layers (C)	1	140‡	291·5‡	565‡	1128‡
Variety (V)	1	73‡	31·7‡	67‡	58·5‡
O×M	35	3·62‡	1·4	3·2‡	5·8‡
O×C	5	—	2·5*	—	—
O×V	5	—	—	—	3·1†
M×C	7	6·68‡	11·7‡	34·7‡	42·8‡
M×V	7	8·99‡	6·9‡	7·6‡	7·1‡
C×V	1	—	—	34·6‡	13·7‡
O×M×C	35	1·39	—	—	3·5‡
O×M×V	35	—	—	—	3·2‡
O×C×V	5	—	—	—	2·3†
M×C×V	7	1·88	—	5·08‡	13·7‡
Residual	35				
Total	191				

* Significant at *P* 0·05.
† Significant at *P* 0·01.
‡ Significant at *P* 0·001.

The water content of the embryos was also used to assess the significance of the effect of oxygen concentration, and of its interaction with other factors, because uptake of water ceases as soon as the embryo is imbibed if germination is delayed (Wellington and Durham, 1961). The values obtained at the four intervals of the test were therefore subjected to variance analysis and it will be seen from Table 2 that oxygen concentration had a highly significant effect only at 120 hours. The water content of the embryos in 40 and 60 per cent. oxygen were then both significantly higher than those in 5 and 10 per cent. oxygen although they did not differ significantly from the values obtained in air or 30 per cent. oxygen. The effect therefore appears to have been due to a reduction in embryo development when the oxygen concentration was lower than normal and not to an increase when it was higher. The stage of maturity, the presence of the covering layers, and the variety, all had significant effects at each interval and there were highly significant interactions between oxygen concentration and maturity. But in the absence of any highly significant interactions between oxygen concentration and the presence of the covering layers, or the variety, there was no evidence that the varietal difference in germination behaviour was caused by differences in the permeability of the covering layers to oxygen.

The relatively small difference between the number of grains with exposed embryos which germinated in 5 per cent. oxygen and the numbers in air, or 60 per cent. oxygen, indicated that the minimum oxygen requirement for embryo development was lower than the concentrations used in this experiment. It was also possible that germination of the intact grains might have been affected by carbon dioxide if this had accumulated within the grain and induced secondary dormancy as in the case of mustard (Kidd and West, 1917). A further experiment was therefore carried out in the following season in which atmospheres of nitrogen, commercial nitrogen containing approximately 0·5 per cent. of oxygen, and air with 3 per cent. of carbon dioxide, were included, in addition to atmospheres containing 5 per cent. and 40 per cent. oxygen, and air as control. The grains were tested for germination at the same stages of maturation, and the same procedure was used for assessing embryo development.

TABLE 3

The Effect of Low Oxygen Concentrations, and the Presence of Carbon Dioxide, on the Development of Exposed Embryos and the Germination of Intact Grains

	Embryos exposed					Covering layers intact				
Germination atmosphere	Weeks after anthesis				Total	Weeks after anthesis				Total
	5	7	9	15		5	7	9	15	
Red variety										
N_2	8	0	0	3	11	0	0	0	0	0
0·5% O_2	7	4	16	9	36	0	0	0	0	0
5% O_2	10	14	25	23	72	0	0	0	0	0
Air (control)	13	25	28	25	91	1	0	1	6	8
40% O_2	14	28	30	25	97	7	1	2	7	17
Air+3% CO_2	11	22	24	29	86	0	0	0	1	1
Total	63	93	123	114	393	8	1	3	14	26
White variety										
N_2	17	4	0	3	24	0	0	0	0	0
0·5% O_2	17	5	15	8	45	0	5	0	3	8
5% O_2	16	26	29	22	93	0	5	13	3	21
Air (control)	19	30	28	26	103	1	14	8	20	43
40% O_2	23	27	31	25	106	3	9	15	20	47
Air+3% CO_2	15	30	25	30	100	0	6	3	12	21
Total	107	122	128	114	471	4	39	39	58	140

The results in Table 3 show that the number of grains with exposed embryos which germinated in air was higher than in the first experiment; and this may have been due to a seasonal difference in maturation. But the same relative differences in germination were caused by the stage of maturity, the presence of the covering layers, and the variety. The fact that some grains of both varieties, with the embryo exposed, germinated in nitrogen must be attributed to oxygen which leaked into the apparatus owing to the difficulty of effectively sealing it. In particular, the values at 5 weeks were obtained before a solution of pyrogallol was used to remove as much oxygen as possible before the nitrogen entered the germination chamber. In spite of the leaks, however, no intact grains of either variety germinated in nitrogen.

In both varieties the number of grains with exposed embryos which germinated in 0·5 per cent. oxygen was approximately half the number which germinated in 5 per cent.; and this was in turn less than the number in air which was almost the same as the number in 40 per cent. oxygen. Some embryos therefore needed very little oxygen in order to germinate and the majority required less than 5 per cent.; but a proportion, which was larger in the red variety than the white, had a requirement approaching the level in air. These might account for the effect of oxygen concentration on the germination of grains of the red variety, with the embryos exposed, in the first experiment.

The presence of the covering layers prevented any intact red grains from germinating in either 0·5 or 5 per cent. oxygen; and as in the first experiment very few germinated in air, although rather more germinated in 40 per cent. oxygen. In contrast a few intact white grains germinated in 0·5 per cent. but there were marked increases in 5 per cent. oxygen, and again in air, and only a further slight increase in 40 per cent. In both varieties the covering layers of some grains were, therefore, sufficiently impermeable to prevent germination when the oxygen concentration in the external atmosphere was very low; and when the external concentration was raised, there was a greater increase in the germination of the white grains than of the red as a result of increased diffusion of oxygen through the covering layers. Since the inhibiting effect of the covering layers of the red grains then remained constant at the higher external concentrations, used in the first experiment, it must be attributed to some factor other than their impermeability to oxygen.

The presence of 3 per cent. carbon dioxide in the air only slightly reduced the number of grains with exposed embryos which germinated in both varieties; but it caused a marked reduction in the number of intact white grains which germinated in air and the small number of intact red grains which germinated was also reduced. It is therefore possible that the presence of the carbon dioxide interfered in some way with the diffusion of oxygen through the covering layers although it did not appear to have any direct effect on embryo development.

DISCUSSION

The most detailed evidence that covering layers, or seed coats, may be sufficiently impermeable to oxygen to prevent germination relates to the seeds of the cocklebur (*Xanthium pennsylvanicum*). Thornton (1935) showed that the embryos of the upper seeds in the fruits required 1·1 per cent. of oxygen, while those of the lower seeds required 0·6 per cent., in order to germinate at 21° C. when they were exposed. But when the covering layers were intact complete germination of the upper seeds was obtained in 60 per cent. oxygen although an atmosphere containing 6 per cent. oxygen was sufficient for the lower seeds. The evidence in other cases is based on an increase in germination when intact seeds have been placed in atmospheres enriched with oxygen, although Toole *et al.* (1956) have pointed out that this does not necessarily prove that the inhibiting factor in air is the slow rate of diffusion through the coats. The availability of oxygen is also increased by exposing the embryos; but when germination is induced by this treatment it may equally be due to the increased availability of water, or the removal of specific germination inhibitors, or the mechanical resistance of the covering layers to embryo expansion. There is no evidence that impermeability of the covering layers to water prevents the germination of wheat grains as it does in the case of 'hard' seeds of many leguminous species (Watson, 1948); nor has a germination inhibitor been found as in the case of *Xanthium* (Waring and Foda, 1957). In previous papers (Wellington, 1956; Wellington and Durham, 1961) evidence has been presented that the covering layers may prevent germination by limiting the expansion of the embryo but the possibility that the covering layers are impermeable to oxygen still remains to be considered.

The numbers of grains with exposed embryos which germinated in the low oxygen concentrations indicate that the minimum requirement for the majority of embryos is rather higher than that found for cocklebur seeds, although it is considerably lower than the concentration in air. But there is also a small proportion of the embryos with a requirement approaching this level and any limitation on diffusion imposed by the impermeability of the covering layers would probably prevent these grains from germinating under normal conditions. The proportion of such grains appears to be rather larger in the red variety than the white but it would only account for a small part of the varietal difference in germination.

The immature grains, at 5 weeks after anthesis, were prevented by the covering layers from germinating in any atmosphere; and at this stage the pericarp consists of several layers of living cells which may impede the passage of oxygen. It has been suggested that this is the reason why the testa prevents the germination of immature peas (Kidd and West, 1920) and it has been shown that living cells in the testa of *Cucurbita* reduced the rate at which oxygen diffused through it (Brown, 1940). In wheat the inhibiting effect on germination, at this stage, is associated particularly with the inner layer of the pericarp which contains chloroplasts and is therefore likely to have considerable metabolic activity. But when the grains are subjected to premature desiccation, which disrupts the layer and destroys the chlorophyll, the inhibiting effect is lost (Wellington, 1956). The gradual increase in the number of white grains which acquire the ability to germinate, as ripening proceeds, may therefore be due to increased diffusion of oxygen through the covering layers when the contents of the cells become disorganised and the tissues are disrupted by desiccation. This would account for the relationship between the effect of the covering layers and the oxygen concentration in the external atmosphere which was obtained for the white grains.

The lack of any corresponding relationship in the case of the red grains is associated with the inhibiting effect of the epidermal layer of the pericarp, which replaces the inhibiting effect of the inner layer as ripening progresses (Wellington and Durham, 1961). Few cells of the epidermal layer appear to have any organized contents at this stage and it is therefore unlikely that their metabolic activity would prevent oxygen diffusing to the embryo. It is possible that some phenol oxidase system may remain active in the cells, and restrict the access of oxygen, as Pollock and Kirsop (1956) have suggested in the case of barley. But this also is unlikely because the effect of the covering layers in the red grains is largely independent of the oxygen concentration in the external atmosphere.

Harrington (1923) has reported that the germination of a freshly harvested wheat sample increased from 44 per cent. in air to 85 per cent. in an atmosphere of 36 per cent. oxygen, but no further increase was obtained in higher concentrations. However, treatment of the grains with sulphuric acid, which removed most of the covering layers, gave prompt and complete germination. In the present investigation the germination of the red grains increased slightly in atmospheres containing 40 and 60 per cent. oxygen but the increment was very much less than that obtained by exposing the embryos. Red grains have also been induced to germinate by removing only the distal end, which would not alter the availability of oxygen to the embryos; whereas they did not germinate when a small portion of the covering layers was removed in the region of the micropyle which would increase the access of oxygen without reducing their mechanical strength (Wellington, 1956). It was therefore argued that the oxygen available to the embryo was not a limiting factor in the germination of the red grains and the different behaviour of the two varieties could be explained by a difference in the resistance of the covering layers to expansion of the embryo. The results of the present investigation indicate that the diffusion of oxygen through the covering layers is not a critical factor in the germination of either variety, under normal conditions, and the varietal difference in germination behaviour cannot be attributed to differences in the permeability of the covering layers to oxygen.

SUMMARY

1. Ears were harvested at different stages during maturation from a wheat variety with red grains and another with white; the grains were then allowed to germinate in atmospheres containing different concentrations of oxygen above and below the level in air.

2. The oxygen requirement of the embryos was determined by the number of grains which germinated when the embryos were exposed and the effect of

the covering layers was obtained by the difference in germination between intact grains and grains with the embryos exposed.

3. In both varieties some embryos required only 0·5 per cent. of oxygen for germination and the majority required less than 5 per cent. But there were also some embryos which required a concentration approaching the level in air and the proportion was rather larger in the red variety than in the white, although this did not account for the varietal difference in germination.

4. The germination of intact white grains showed a marked gradient with increasing oxygen concentration up to the level in air but there was only a slight increase in the germination of intact red grains.

5. The inhibiting effect of the covering layers in the white grains was reduced when the oxygen concentration was increased, but the effect of the covering layers in the red grains remained approximately constant. The varietal difference in germination cannot, therefore, be attributed to differences in the permeability of the covering layers to oxygen.

ACKNOWLEDGEMENTS

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The Change of Tropism in *Polyporus brumalis* Stipes and the Effect of Directional Stimuli on Pileus Differentiation

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With Plate and 5 Figures in the Text

ABSTRACT

The directional influences of light and gravity upon the development of pileate and epileate sporophores of *Polyporus brumalis*, produced in pure culture, have been investigated. Growth in the dimitic fruit-body has a considerable sub-apical component which in the stipe is responsible for tropistic curvature. The stipe is competent to react negatively to unilateral gravitational and positively to unilateral photic stimulation throughout development, but when both stimuli operate phototropism masks geotropism. If illuminated from one side the growing epileate stipe is strongly positively phototropic but as the pileus reaches a characteristic diameter the stipe becomes negatively geotropic. Experiments with changing direction of illumination and with artificial pilei of black paper suggest that the change of tropism is explicable by the shading action of the expanding pileus on the sub-pileal photoperceptive and photoreactive region of the stipe.

Developing sporophores continuously rotated with reference to fixed directions of gravitational and light stimulation and others with stipes inverted as a result of illumination from below during development have in common that the morphologically upper surface of the pileus always develops towards and approximately at right angles to the direction from which the maximum light intensity is received. Normal but inverted stipes and dissepiments show no tendency to geotropic reorientation.

INTRODUCTION

RECORDED phototropic responses in hymenomycete sporophores would seem to be confined to stipes, whereas geotropism has been invoked to account for the direction of growth of some pilei and of most stipes and hymenophores.

Stipes of *Agaricus campestris* (Buller 1909, Chap. IV) and probably many other ground agarics are at all stages nonphototropic, and those of others such as *Amanita phalloides*, *A. crenulata* (Streeter, 1909) and the coprophilous *Coprinus sterquilinus* (Buller 1930, p. 121, Jeffreys and Greulach, 1956) are only mildly light-responsive, seldom turning more than 10° from the vertical when unilaterally illuminated. Probably the stipes of all ground agarics, however, have a marked negative geotropism demonstrable by tilting the developing fruit-body and observing its corrective movements.

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In contrast stipes of lignicolous and coprophilous hymenomycetes often have strong phototropic as well as geotropic competence. These tropisms may be important for the emergence of the developing sporophore from recesses and for the positioning of hymenophores (Buller, 1909). The two kinds of response are separated in time. An initial period of light-seeking growth is followed by negative geotropism. In an agaric with aphyllophoralean affinities, *Lentinus lepideus* (Buller, 1905, 1909), the initial phototropism is characteristic of the as-yet-epileate stipe, whereas in more typical agarics it occurs in stipes which already bear rudimentary or incompletely expanded pilei [*Coprinus niveus*, *C. curtus* (Buller, 1909, Chap. IV+XXI), *Coprinus lagopus* (Borriss, 1934*b*)]. Once the cap has formed, or considerably enlarged, a process usually itself requiring light as a morphogenetic stimulus, a spectacular change occurs: the stipe curves away from the centre of the earth and growth appears no longer to be affected by the direction of illumination. Buller suggests that this sequence of events occurs in *Lentinus lepideus* because 'a remarkable change takes place in the physiological properties of the stipe' (1909, p. 48).

In the pilei of woody Aphyllophorales, which mainly grow at the extreme margin only, it has been suggested that approximate horizontality is attained by dia- or plagio-geotropic growth (e.g. *Fomes fomentarius*, Buller, 1922, Chap. IV). Presumably such a gravitational sensitivity would reside in the hyphal apices forming the marginal meristem. The principal evidence supporting the supposed gravitational influence comes from developing sporophores which have been reorientated by tree-fall or log rolling. New growth occurs in an approximately horizontal position irrespective of the orientation of the previously formed parts of the pileus [*Fomes fomentarius* (De Jaczewski, 1910; Massart, 1920; Buller, 1922) *Fomes pinicola* (De Jaczewski, 1910) *Lenzites deplanata* (Massart, 1920).] Such a treatment would alter the relationship of the meristem to other spatially asymmetrical stimuli as well as gravity and really adds very little to the initial observation of horizontality in undisturbed fruit-bodies.

It has not been claimed that agaric pilei show any geotropic growth. They are probably brought into an approximately horizontal position by the growth movements of the stipe (Buller, 1909). Indeed it is a matter of observation that the pilei of many agarics, in the course of ripening and during spore discharge, move from a deconic through a flattened to an obconic shape. The motive force of this movement is probably provided by the relatively greater swelling of the hyphae of the gills and lower side of the cap than those of the upper surface of the cap (*vide Collybia apalosarca*, Corner, 1934) and any gravitational response would have to operate through this mechanism. A similar situation obtains in *Polyporus squamosus*, a polypore in which swelling of tissue behind the under-rolled margin plays a large part in pileus growth (Corner, 1953). Here, however, diageotropism of the cap has definitely been claimed by Buller on the basis of the position assumed by the growing parts of pilei kept in darkness following displacement from their former orientation (Buller, 1906; Buller, 1909, p. 63).

Hymenophores are usually positively geotropic. It was Buller's most memorable contribution to the biology of the hymenomycetes that he showed efficient basidiospore liberation to call for a downward facing or vertical hymenium in the majority of species. It followed that the wedge-shaped or parallel-sided hymenophores should be positively geotropic. This compelling teleological argument distracts attention from the paucity of published evidence. There is no doubt, however, that hymenophores are observably vertical and that in many fleshy agarics, including *Agaricus campestris* (Buller, 1909, Chap. IV), geotropic adjustment occurs by differential inflation of the hyphae at the base of the lamellae when they are tilted from the vertical. Mature hymenophores of woody and leathery sporophores cannot change their orientation with relation to the pileus but any new growth after the displacement of a fruit-body, or part of it, will have its dissepiments in the new vertical and downward position [*Fomes fomentarius* (Buller, 1922; De Jaczewski, 1910; Massart, 1920), *Fomes marginatus*, &c. (Lohwag, 1949), *Ganoderma lucidus* (Bose, 1940), *Polystictus versicolor*, *Lenzites deplanata* (Massart, 1920), *L. sepiaria* (Kreh, 1925), *L. betulina* (Lohwag, 1955).]

The qualitative experiments with *Polyporus brumalis* reported in this paper are here communicated as offering a novel explanation of changing tropistic behaviour in the stipe and preliminary information on pileus differentiation and geotropic mechanisms in stipe and hymenophore.

METHODS

The basic technique used to produce sporophores of *Polyporus brumalis* in pure culture is described in an earlier paper (Plunkett, 1956). In more recent work the practice of adjusting the 4 per cent. malt extract medium to an initial pH of 4·2–4·3 has been adopted because of the sensitivity of the fruiting process to H-ion concentration and the variability in acidity of different batches of malt extract.

The present investigations called for a comparison of the tropisms of epileate and pileate stipes. Use was made of the fact that pileus production can be controlled at the experimenter's convenience. Pileate fructifications were produced in streams of dry sterile air flowing through the glass culture chambers (i.e. large dessicator jars) at 200 ml./min., with illumination from fluorescent-tube lamps of 60–100 f.c. When it was desired to suppress pileus production light intensities of 40–75 f.c. were used and a moist air stream (*c.* 96 per cent. R.H.) passed at 5 ml./min.; or, more simply, a few hundred ml. of water was placed in the bottom of the culture chamber below the shelf of the desiccator and the atmosphere was not renewed. As water losses by evaporation were negligible under these conditions it was usually unnecessary to use the automatic water-replacing device (Plunkett, 1956) required in rapidly transpiring cultures. In moist chambers it was also possible to increase the number of culture vessels per chamber above the five previously used.

The experiments were performed at laboratory temperature which varied little from 18° C. It is known that over a wide range (<10°–>27° C.) temperature affects the rate but no other obvious aspects of development.

It was found that there was no apparent development of contaminating organisms on exposure to the laboratory air once the mycelium had covered the surface of the culture vessels. This allowed great freedom of manipulation during the subsequent fruit-body development.

Other relevant details are given in connexion with individual experiments.

GROWTH REGIONS AND HYPHAL CONSTITUTION

No detailed study of sporophore growth is presented here, but a few preliminary observations may be recorded which are not without relevance to the main subject.

Five sporophores maintained as epileate stipes by the use of moist culture atmospheres and fairly low light intensities (*c.* 40 f.c.) were marked with indian ink at 1 mm. intervals from the apex. They were subsequently withdrawn from the culture chamber once per day and the lengths of the marked sections remeasured. For this a horizontal microscope moving over an accurately graduated vertical-scale was employed.

From the diagram (Fig. 1) which shows diagrammatically the means of the daily increments in each stipe section it is clear that under the specified conditions linear elongation, although slightly irregular, amounted to 2 to 3 mm. per diem. Growth was confined to the terminal 5 mm. and was considerable only in the terminal 3 mm. The two segments of the stipe behind the apical segment underwent a slightly greater than twofold enlargement during the first 2 days. Over the same period about three-fifths of the total elongation of the stipe was due to the growth of that part which lay behind the terminal 1 mm. at the time of marking.

Current work, to be published in detail later, shows that the growth-rate of stipes in dry atmospheres can be as great as 10 mm. per day. If it is assumed again that only 5 mm. of the stipe of such fruit-bodies is extensible, the amount of growth after pileus initiation suggests that the subterminal flesh may elongate as much as five times. Similarly the separation during development of ink-markings made on young pilei under various conditions indicated that 3·3-times enlargement was taking place (mean of 14 determinations). It may be concluded that growth has apical and sub-apical components and that the latter ['inflation' in Corner's (1934) terminology] plays a not inconsiderable part in total growth.

A few simple anatomical studies have been made by teasing apart thick longitudinal sections of various regions of sporophores in a drop of 5 per cent. KOH under a dissecting microscope. The fungus is clearly dimitic. It consists of thin-walled generative hyphae and thick-walled and fairly richly branched binding-hyphae not unlike those found in *Polyporus squamosus* (Corner, 1953). Both are present in most parts of the mature fruit-bodies; but in epileate stipes, the only developing stage carefully investigated, the binding hyphae do not extend into the terminal regions but are to be found first at about 5 mm. behind the apex. Thus the growing region of the stipe is composed solely of sappy, thin-walled generative hyphae. Growth in length might be expected to cease in the older and tougher portions of the sporophore if only because of the invasion of the flesh by the thick-walled and essentially inextensible binding hyphae.

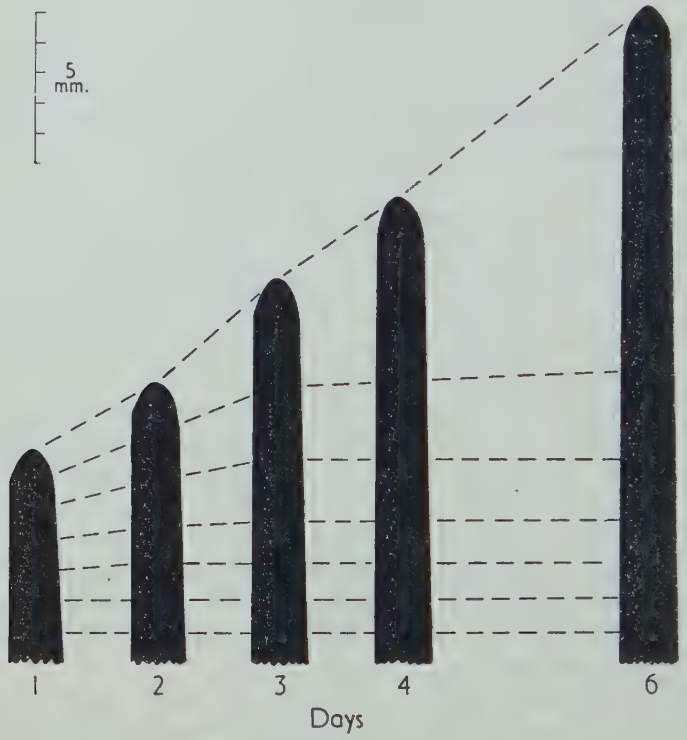

FIG. 1. The subsequent growth of 1 mm. segments of stipe marked on day one (means of 5 fruit-bodies). Evaporating conditions slow, light intensity *c.* 40 f.c.

EXPERIMENTS ON THE TROPISM OF STIPES

1. Phototropism in Epileate Stipes

The phototropism of epileate stipes has received earlier mention (Plunkett, 1956, Pl. 28, Fig. 1). It is a strong reaction so that the stipe apex comes to point directly at a unilateral light source (Fig. 2) even if this involves a curvature through nearly 180°. Although the minimum amount of light required to activate the phototropic mechanism has not been determined, continuous illumination of an intensity of 1 f.c. at the fungus surface evokes some response. With continuous light at the intensities used in the experiments of later sections 24 hours was always sufficient for a 90° curvature. A very brief exposure to strong unilateral illumination is sufficient to cause positive curvatures.

Twenty epileate stipes which had been growing upright in moist chambers with weak top illumination were irradiated from one side at an intensity of 1,500 f.c. Exposure periods varied from 12 to 300 seconds. The stipe was marked almost at the top but on the illuminated side with a spot of indian ink and the culture replaced in a moist atmosphere in complete darkness. After 24 hours all stipes displayed a curvature towards the illuminated side which was measured with a protractor and varied from 5° to 80°. The curvatures lay behind the indian-ink mark thereby revealing the motor region of the response. Plate, Fig. 2 shows two fruit-bodies which developed a curvature of 50° and 35° respectively following an exposure of 240 seconds; it shows also the sub-terminal motor region.

It is noteworthy that curvatures developed although no attempt was made to remove the effect of a possible geotropic potential (see below). A few sporophores which were similarly treated but replaced in chambers with weak top light instead of darkness failed to curve during the experimental period.

No attempt was made to prevent possible heat radiation from the light source, although the brevity of some exposures make it unlikely that it had any influence.

The latent period and period for maximum response were not investigated.

2. *Development with Unilateral Illumination, changing Tropistic Behaviour*

Erect epileate stipes were produced by weak top-illumination together with the slow passage of moist air. When the stipes were about 5 mm. high the entire culture chamber was placed inside a wooden box which lacked one side. Air- and water-supply tubes entered through the top of the box. The open side was then screened with black cloth so that light was admitted only at the level of the fruit-bodies by a wide horizontal slit the full width of the box and 5 cm. deep. A horizontal fluorescent-tube lamp was placed parallel to the opening at an average distance of 20 cm. from the stipes which received thereby light at an approximate intensity of 75 f.c.

After 24 hours all the sporophores showed a positively phototropic curvature through 90° and were growing directly towards the light source (Fig. 2, *1*, *2*). At this point dry air was passed through the chamber at 200 ml./min. which in combination with the given light intensity could be expected to induce pileus formation. This followed in about 2 days, the caps extending at right angles to the direction of the light source and hence to the long axes of the stipes. The stipes continued to elongate without change of direction until the pilei were 2 to 3 days old and about 9 mm. in diameter (Fig. 2, *3*; Plate, Fig. 6). Thereafter the stipes began to bend upwards and in 2 days their upper parts were more or less vertical and the pilei, now about 18 mm. wide, approximately horizontal (Fig. 2, *4*; Plate, Fig. 3). For the sake of simplicity this account has described the pileus as though it were perfectly flat. In fact up to this stage it is slightly epinastic but in the next few days flattens or becomes upwardly concave.

The final position of the stipe was usually vertical but sometimes showed a slight departure from this orientation as though the geotropic corrective movement were incomplete (Plate, Fig. 3). The possible causes are considered in the discussion.

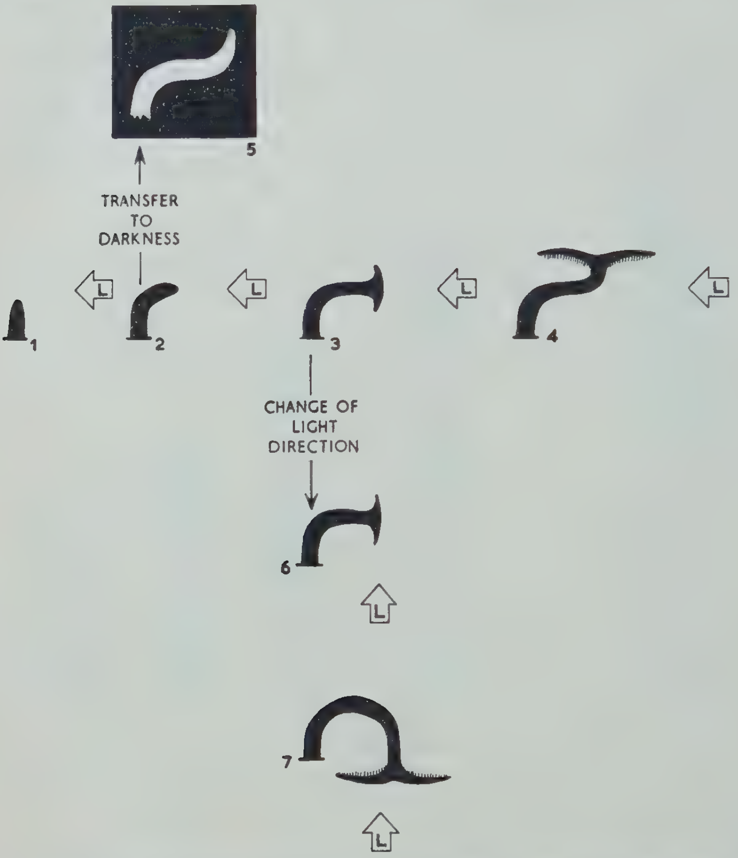

Fig. 2. Development of sporophores: 1–4, illuminated horizontally from one side, showing successive phototropic and geotropic curvatures. 5, Geotropic curvature following transfer to darkness of stage 2 fruit-body. 6–7, Phototropic curvature following change of direction of light source from the side to below at stage 3. Diagrammatic. The arrows marked 'L' indicate the direction of illumination in this and subsequent figures.

With small differences in timing and dimensions three fruit-bodies studied in a first experiment and three in a repeat all developed in the way described.

Thus young unilaterally illuminated sporophores of *P. brumalis* develop in such a way that the stipe is at first positively phototropic and then negatively geotropic, the position of the light source being fixed. As in *Lentinus lepideus* (Buller, 1905, 1909) the change in direction of stipe growth is associated with the development of the pileus. It is of the highest significance, however, that the change does not occur until the cap reaches a characteristic diameter (about 9 mm.) at the light intensities studied.

3. Geotropism of Epileate Stipes

The behaviour just described raised the question of whether the epileate stipe was exclusively phototropic and the pileate stipe exclusively geotropic. The epileate stipe was investigated first, using the simple culture technique applicable when no pilei are required (see methods).

Ten young stipes were unilaterally illuminated so that they curved phototropically and grew horizontally for about 1 cm. They were then placed in darkness in a moist chamber and inspected after 4 days. All had made a negatively geotropic growth correction so that the top of the stipe was again vertical (Fig. 2, *5*).

In a second experiment more than 10 vertical stipes, 1 to 1·5 cm. long, were developed upon a medium to which 2 per cent. agar had been added, a permissible practice with this fungus when evaporation rates are low. The tips of the stipes were ink-marked and the culture flasks and hence the stipes were arranged in a horizontal position, possible because of the solid medium, in a completely dark moist-chamber. Inspection after 4 days showed that the stipes had curved through 90° to adopt the new vertical position. As in the case of phototropism the position of the ink marks revealed that most of the curvature had occurred in the part of the stipe formed before the treatment was applied (Plate, Fig. 5).

Thus the epileate stipe, whether or not it has already undergone a phototropic readjustment, is fully competent in darkness to perceive and react to a gravitational stimulus.

It is evident that in illuminated cultures this geotropic reaction potential is masked by a stronger phototropic reaction.

4. The Phototropism of Pileate Stipes

Attention was next turned to the pileate stipe in that phase when it normally shows negatively geotropic growth.

The culture conditions described in a previous section (p. 211) were employed to produce sporophores in which the distal parts of the stipes were horizontal and bore young pilei of just under 10 mm. diameter (Fig. 2, *3*). At the point when it was expected that negatively geotropic curvature would appear the culture vessels were transferred to another culture chamber so arranged that the sporophores were illuminated from below instead of horizontally. This was achieved by replacing the shelf of a normal culture chamber by a glass plate and standing the whole assembly on a plate-glass platform about 20 cm. above a fluorescent-tube lamp. The fungus received light at an intensity of *c.* 75 f.c. Light from directions other than below was excluded by draping the culture chamber with several layers of black cloth. A fan was used to prevent the accumulation of heated air above the lamp. As caps were already differentiated it was unnecessary to apply rapid evaporating conditions for the 2 to 3 days required to complete the experiment. Unrenewed air in contact with a few grams of anhydrous calcium chloride ensured that conditions were not excessively moist but did not make the replacement of water loss obligatory.

The result of the treatment was spectacular. Of the seven fruit-bodies treated as they reached the appropriate stage of development all executed a downward instead of an upward curvature. This made the distal part of the stipe approach the vertical downward and the morphologically upper surface of the pileus was approximately downward-facing (Fig. 2, *7*; Plate, Fig. 1). The failure of some stipes to become completely vertical is considered in the discussion. Three sporophores maintained in the original vessel with horizontal illumination and similar evaporating conditions were employed as controls. They, as expected, displayed a negatively geotropic curvature.

The experiment demonstrates plainly that the stipe remains capable of positively phototropic curvature even after the cap has reached the critical size. Here too the phototropism is a stronger response which masks geotropic potential under the conditions of the experiment, that is while the stipe is strongly illuminated. Comparison with development in the presence of fixed horizontal illumination from one side makes it clear that the photoperceptive area lies behind the pileus (compare *3* and *4* with *6* and *7* in Fig. 2). There is a strong suggestion that the intensity of light falling on the immediately subpileal part of the stipe determines whether phototropism or geotropism is manifested and that the usual upward curvature in fruit-bodies developing with side illumination is simply due to the *shading* of the photoperceptive region by the developing pileus.

The next experiment amply supports this conclusion.

5. *An Experiment with Artificial Pilei*

If the change from phototropic to geotropic behaviour in stipes is due to the shadow cast by the developing pileus it should be possible to bring about a similar change in epileate stipes by reducing the light intensity incident on the growing photoreceptive region. This was done with artificial 'pilei' of black paper.

Low evaporating conditions in combination with fixed unilateral illumination yielded epileate stipes with horizontally growing distal portion as in previous experiments. The horizontal slit in the side of the box admitting the only light to the cultures was reduced to 2 cm. in depth.

Black paper discs 18 mm. in diameter were cut with a cork borer. Using a tiny drop of sealing-wax the end of a length of fine, inflexible wire was joined marginally and at right angles to the disc. The other end of the wire was bent through 90° and fashioned into a terminal loop which would fit tightly on a stipe about 10 mm. behind its apex. By tailoring each attachment wire to fit a particular stipe it was possible to interpose a disc at right angles between the stipe and the light source with the centre of the disc exactly opposite the apex and about 5 mm. away from it (Fig. 3, *2*). Great care was exercised to avoid damage to the fungus. By means of reference marks on all movable parts of the apparatus the original orientations of the stipes to the light source were re-established following the fitting of the pseudo-pileus and later inspections. This delicate experiment was performed with eight fruit-bodies in all. They responded with a high degree of consistency.

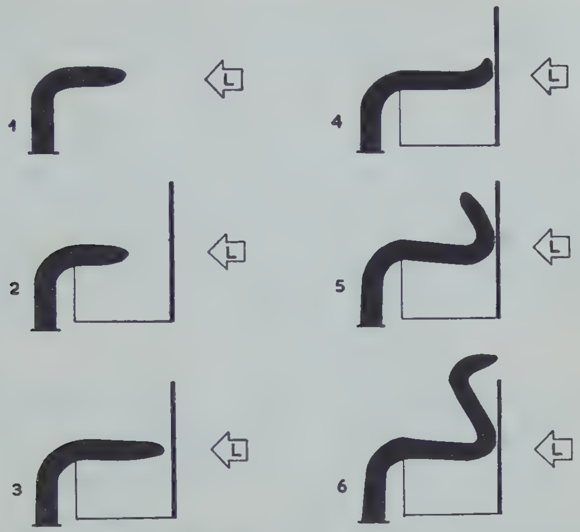

FIG. 3. The effect of interposing a disc of black paper (attached to the stipe by wire) between the light source and the stipe apex. 2–3. Straight growth. 4. Negative geotropic curvature 0·5 mm. behind the disc. 5. Second phototropic curvature. 6. Third phototropic curvature. Diagrammatic.

Stipes continued to grow in the original direction for 2 days until they were within about 0·5 mm. of the centre of the artificial pileus. At this point an upward curvature occurred (Fig. 3, *3* and *4*). The delay in response indicates clearly that this is not a phototropic curvature which would have been clearly manifested in 24 hours (see Section 1, p. 210) and at a greater distance from the paper disc. Furthermore all eight specimens curved in the same direction. It must be considered extremely unlikely that any chance asymmetry in illumination would in all cases have been biased in the same direction. The movement can thus be interpreted as negatively geotropic and is strikingly associated with the position of minimum light intensity a fraction of a millimetre behind the centre of the paper disc.

As the stipe approaches the paper it grows down a gradient of light intensity. A regular increase of light intensity must also occur along radii at right angles to any point on the long axis of the stipe. The long initial period of straight growth is the best indicator of the symmetry of this illumination. After its upward curvature, however, the apical part of the stipe will receive more light on the side towards the stipe base. Therefore it is not surprising that the next response, observed at about 3 days from the beginning of the experiment, was growth backwards from the centre of the paper disc (Fig. 3, *5*; Plate, Fig. 4). The actual direction, about 40° from the vertical, was presumably determined by a path of most rapid increase of light intensity, a resultant of the longitudinal and transverse gradients already mentioned. As the tip approaches the margin of the pseudo-pileus the direction of the maximum photic stimulus changes rapidly. Thus the last directional response of the stipe was a positively phototropic curvature bringing its terminal part into direct alignment with the direction of the light source once more. This occurred some 6 to 7 days after the beginning of the experiment (Fig. 3, 6; Plate, Fig. 4).

The results show again the dual tropistic competence of the stipe and that geotropism is only dominant under conditions of low light intensity when it may be presumed that the phototropic mechanism is understimulated. The fact that a pileus-like disc physiologically unconnected to the stipe can cause the change from photo- to geotropic behaviour strongly suggests that the true pileus similarly acts in a simple fashion—that is as a light screen.

PILEUS DIFFERENTIATION

The remaining experiments are concerned with stipe tropisms as well as pileus differentiation, a subject of current investigations in this laboratory.

1. Pileus differentiation in unilaterally illuminated sporophores

On a photically induced horizontal stipe a pileus differentiates at approximately right angles to the direction of the incident light (and the long axis of the stipe) until it is nearly 1 cm. in diameter (p. 211 above). As much of the enlargement of the cap is due to inflation of hyphae lying behind the margin of the pileus (see section on growth regions) possibly most or even all of the apical extension of hyphae in the edge-meristem is completed by this time. Evidently the mechanisms whereby the tissues of the pileus are laid down are in no sense dependent on its being horizontally orientated.

When the negative geotropism of the stipe carries the pileus into its final horizontal position the pore-field is sketched out on the under surface of the cap, but the hymenial tubes are still exceedingly shallow.

2. Klinostat experiments

Cultures of *Polyporus brumalis* on 4 per cent. malt extract 2 per cent. agar medium filling 100 ml. crystallizing dishes were rotated on a klinostat from the time of inoculation, the klinostat disc and agar surfaces being parallel. Until the mycelium covered the agar the dishes were kept free of contaminants by temporary lids held in position by adhesive tapes. Subsequently a non-gas-tight, cylindrical perspex chamber, sterilized inside by mopping with 70 per cent. alcohol, was attached to the disc and enclosed the cultures. The culture atmosphere was kept moist by the addition of small amounts of sterile water until fruit-body rudiments appeared, when it was allowed to become moderately dry by gaseous exchange with the laboratory atmosphere. Under these conditions well-developed pilei were often but not invariably produced. The results given relate to cases where no obvious arrest of development occurred.

As pileus production is light-dependent, illumination was provided from a fixed source (100 watt tungsten-filament lamp) outside the rotating chamber. Different directions of illumination were employed in different experiments (Fig. 4 *a*, *b*). In one the light-source hung above the klinostat so that the direction of light was parallel to the agar surface. In the other the lamp was placed beside the klinostat so that the direction of the source was perpendicular to the agar surface. In each case a black-out was devised to exclude light from undesired directions (Fig. 4 *a*, *b*).

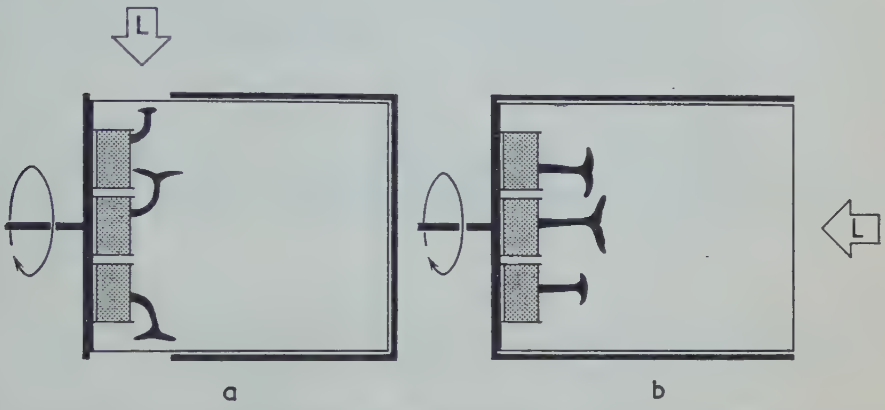

FIG. 4. Effect of rotating cultures with reference to the direction of the gravitational pull (downward) and a fixed light source, (*a*), parallel to, (*b*), perpendicular to the klinostat disc and the agar surface. Diagrammatic. Heavy lines round the klinostat chamber represent blacked-out surfaces.

When the direction of the light was parallel to the agar surface the stipes curved so that their distal parts grew parallel to the radius of the klinostat wheel above which the stipe originated and always away from the wheel's centre. This is the direction in which, despite the turning of the wheel, the stipe apex will receive the greatest amount of light and, intermittently, the greatest light intensity possible in the circumstances. The special case of a sporophore arising exactly over the centre of the wheel did not occur. The pilei differentiated in their normal morphological relationship to the stipe despite the changing direction of the gravitational pull and the light source (Fig. 4*a*). These observations, which relate to six fruit-bodies, were made several years ago. Upper and lower surfaces of the cap were readily distinguished but unfortunately no investigation of the extent of development of the dissepiments was made.

When the direction of the light was perpendicular to the agar surface the stipes grew without curvature in a direction parallel to the axis of rotation of the klinostat. Again the pilei differentiated at approximately right angles to the long axis of the stipe (and to the direction of the light) with the morphologically upper surface towards the light source (Fig. 4*b*). In the two fruit-bodies studied extremely shallow hymenial tubes, 1 mm. deep, were produced but developed no further, whether because of the deliberately imposed conditions or others, such as water-lack, is not known. Fertile basidia were present in sufficient numbers to give a faint spore print when a pileus was placed on paper.

Indifference of pileus development to changing directional stimuli of light and gravitation would seem to be shown by these experiments; but it is noteworthy that the morphologically upper surface of the pileus again formed in such a way that it was perpendicular to the direction from which the greatest light intensity was received.

3. *Inverted fruit-bodies*

The development of sporophores with illumination from below proved instructive concerning development in hymenophores as well as stipes and pilei.

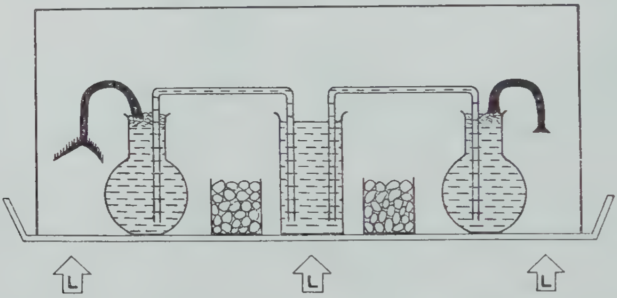

FIG. 5. Inverted fruit-bodies developing in response to illumination from below through the glass supporting tray. The culture chamber, darkened from the sides and above, contains culture flasks connected by siphons to a central reservoir of sterile water. The two small vessels contain anhydrous $CaCl_2$.

The culture assembly consisted of a group of five culture flasks part filled with liquid medium and connected by siphons to a central reservoir of water (cf. Plunkett, 1956). These stood on a rimmed glass tray and were covered by an inverted glass trough. After autoclaving, and as it became necessary later, sterile water was added manually to the reservoir. The full flasks were inoculated. Water added to the glass tray entered the chamber and ensured that the internal atmosphere was moist. Top illumination was provided until the epileate stipes were about 0·5 mm. long. They were then illuminated from one side for 1 to 2 days to cause a 90° curvature. Finally the inverted trough was replaced by a light-tight tin of similar dimensions, blackened inside, and the cultures were illuminated from below at an approximate intensity of 75 f.c. (Fig. 5). The stipe curvature continued until they were growing directly downwards. When growth in this direction had reached a length of 1 cm. or more increased evaporation conditions were applied by the introduction of small vessels of anhydrous $CaCl_2$ into the chamber. In some experiments fruit-bodies had ceased to develop at this stage but in others pileus production occurred.

The caps produced had upwardly opening hymenial tubes and the morphologically upper surface of the cap was downward-facing. In short a reversal of the normal dorsiventrical polarity of the pileus occurred with reference to its surroundings but not with reference to the stipe (Fig. 5). The normal, fully developed but upwardly opening hymenial tubes were fully fertile. Fifteen sporophores have now been induced to complete their development in this culture assembly. None has shown any other orientation.

Thus it appears that there are two null-positions for gravitational curvature of pileate stipes (growing with fixed unilateral light-source). The obvious one of these is when the stipe is vertically upright as a result of illumination from above. Gravitational and photic stimuli are aligned and the pileate stipe does not curve. Another and previously unsuspected null-position is shown by the present experiment. This is when the stipe grows vertically downward as a result of illumination from below and, despite pileus production, remains upside-down.

Similarly it is clear that dissepiments may differentiate freely in an upward as well as the familiar downward direction.

The simplest interpretation of these observations is that graviperception does not occur or that the gravitational response mechanism is unstimulated in vertical stipes and tubes.

In common with the variously orientated caps mentioned in the previous two sections the morphologically upper surface of the pileus in this experiment again developed towards and at right angles to the direction of, the light source. It is tempting to suppose that the mechanism of pileus differentiation makes this obligatory.

DISCUSSION

The results seem to establish that the successive phototropism and geotropism of the *P. brumalis* stipe is due not to some sudden change in sensitivity to stimuli or loss or gain of tropistic mechanism but to the simple effect of the shading of the photoperceptive part of the stipe by the expanding pileus. This may be expected to result in the under-stimulation of the phototropic mechanism so that the geotropic process becomes dominant. Such an interpretation is justified by the fact that the upper part of the stipe remains capable of phototropic curvature, if suitably illuminated, at a time when it would usually curve upwards in response to gravity. Further the shading of an epileate stipe apex by an artificial pileus having no physiological connexion with the stipe results in a curvature that can only be regarded as negatively geotropic (see page 215). It is predictable that a similar curvature would occur if the epileate stipe were placed in diffuse light of sufficiently low intensity, just as it does in darkness. It might be asked why a pileate stipe making its final geotropic correction (when it has fixed illumination from one side) does not respond to light a second time as the upper part of the stipe is re-exposed to strong light. One reason seems to be that the growth potential of the stipe is exhausted by this time. It is to be remembered that sub-apical growth is not unlimited but probably depends on the amount of inflation possible in the cells and the rate of invasion of the growing tissue by inextensible binding hyphae. From the time that the pileus is initiated until it reaches the characteristic size for geotropic stipe curvature to occur occupies about 2 days. A further 2 days is taken for the geotropic adjustment. The total of about 4 days corresponds approximately to the period during which sub-terminal parts of stipes continued to elongate in the straight-growth experiments illustrated in Fig. 1. It was observable too that the final position of stipes sometimes departed a little from the vertical (Plate, Fig. 3), which could be interpreted in at least three ways: (1) as due to exhaustion of growth potential before reaching the vertical, (2) as due to a little phototropic back-curvature after reaching the vertical, (3) as due to sagging of the lower part of the stipe which is subject to much leverage due to the weight of the pileus. Observations at short time intervals would help to distinguish between these possibilities and would also settle whether oscillation about the vertical position happens in the manner reported for *Coprinus plicatilis* and *C. curtus* (Buller, 1909) and *Amanita spp.* (Streeter, 1909). As verticality is approached the growing region must be very small and extremely near to the pileus. It would thus remain shaded until the stipe was quite vertical, especially as the pileus margin is slightly epinastic until just about this stage. If a phototropic back-curvature occurred it would almost immediately cease as the pileus again shaded the growing region.

The phototropic curvature downward of pileate stipes (p. 213) is similarly characterized by the failure of some stipes to reach the exactly vertical position (Plate, Fig. 1). This is most probably due to the shading, as curvature occurs, of the photosensitive region by the pileus with a consequent diminution of the phototropic reaction. A negatively geotropic back-curvature might also tend to be developed.

It is integral to the proposed mechanism of changing tropistic behaviour that the photoperceptive region for phototropic response should lie behind the pileus. If it resided in the upper surface of the pileus the stipe might be expected to continue to elongate towards the light source even after the pileus had reached its critical diameter. This does not occur except in the two special geotropic null-positions. No attempt was made to determine the extent of the photoperceptive and photoresponsive zones. In *Coprinus stiriacus* (Knoll, 1909) and probably *Coprinus sterquilinus* (Jeffreys and Greulach, 1956) they are coextensive and lie behind the pileus.

Whereas the stipes of *P. brumalis* and *Lentinus lepideus* (Buller, 1909) become pileate only a short time before the end of the phototropic phase, a pileus rudiment appears long before this in the strongly phototropic agarics [*Coprinus niveus*, *curtus* (Buller, 1909), and *lagopus* (Borriss, 1934*b*)]. The pileus is, however, small and it is likely that a considerable part of the growing region of the stipe is still not covered by it (see the data of Borriss for *C. lagopus*, 1934*a*). The onset of negative geotropism is associated with an enlargement of the pileus and a shift of the lower edge of the growing region towards the top of the stipe so that shading of the sensitive region probably occurs. It is thus possible that a mechanism of change of tropism similar to that in *P. brumalis* could operate in such agarics.

The experimental results were suggestive concerning pileus differentiation. This is a process apparently indifferent to the direction of the gravitational stimulus. Under a wide range of conditions the cap always spreads at right angles to the direction from which the strongest light intensity is received, that is, it may be diaphototropic. It could equally well be said, however, that it grows at right angles to the stipe which, being a positively phototropic structure at this stage, would ensure the same orientation. A considerable amount of current work concerned with pileus differentiation has in no way contradicted the possibility of a diaphototropic pileus.

A further feature of pileus differentiation was that the morphologically upper surface was always directed towards the strongest light intensities, that is to say the hymenial tubes were always on the least illuminated side. This occurred despite a reversed or continuously changing relationship to the direction of the gravitational field. It is of interest to note that a report of exceptional fruit-bodies with hymenophores on upper as well as lower surfaces of pilei relates to sporophores which had grown at reduced light intensities (Maheu, 1926). Buller, 1900 (Chap. 4) records such a case in *Polyporus squamosus* growing in weak light. The present writer has collected a sporophore of the same species in nature in which hymenial tubes occurred on the upper surface of a pileus deeply shaded by another normal cap. It has been said, teleologically, that the pileus of a hymenomycete sporophore is an umbrella which protects the hymenium from disturbance by rain (Corner, 1944). To continue such speculation the pileus may equally well be likened to a parasol which protects the hymenium from radiant heat and ultraviolet light. In nature rain and the strongest light tend to come from the same quarter so that natural selection for protection of hymenium against either rain or radiation could favour the evolution of a diaphototropic pileus with light-intensity determined upper-sterile, lower-fertile dorsiventrality.

The belief that stipes are negatively geotropic and dissepiments positively geotropic, almost an article of faith with mycologists since Buller's day, might seem to be called in question by the experiments in which fully inverted fruit-bodies were produced. In fact the stipe is not positively as well as negatively geotropic. If placed horizontally at sufficiently low light intensity the stipe always turns upwards and not downwards. This fact taken in conjunction with the failure to respond geotropically in the inverted position after pileus formation gives preliminary information on the geotropic process. Thus the stipe may require some departure from the vertical to start the negatively geotropic machinery rather than being positively attracted to the vertical pathway.

Dissepiments of *P. brumalis* also grow with equal facility in either of the two vertical directions. Here the situation is less easy to interpret as the structures cannot undergo tropistic reorientation once formed. However, their growing margins may be under the influence of gravity and like the stipe hyphae may have two null positions for geotropic stimulation. It is desirable that the klinostat experiments should be repeated with special attention to hymenophores. A fundamental reconsideration would be necessary if they were capable of full development in a horizontal position. On teleological grounds it would seem an unlikely happening, a view reinforced by the almost invariable verticality of dissepiments in nature.

ACKNOWLEDGEMENT

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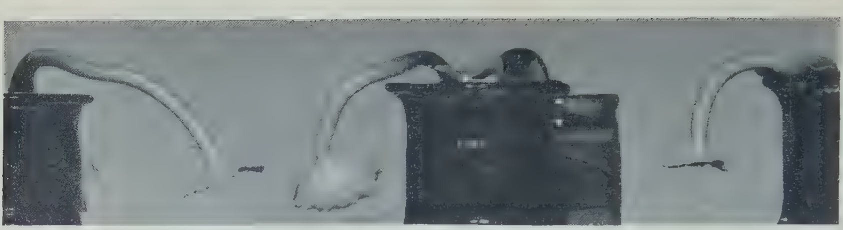

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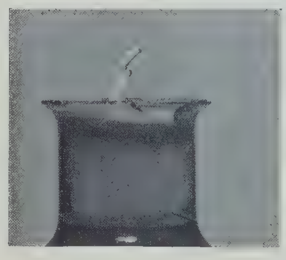

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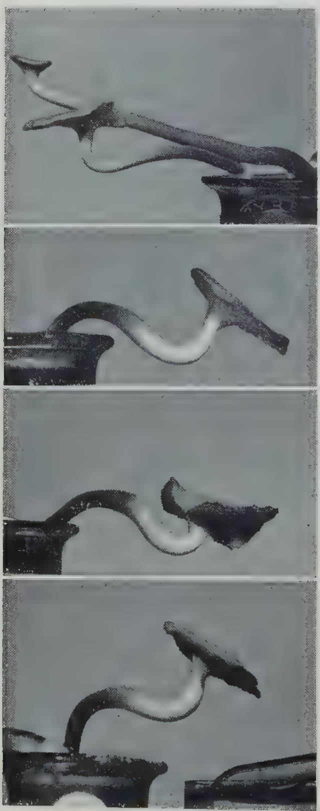

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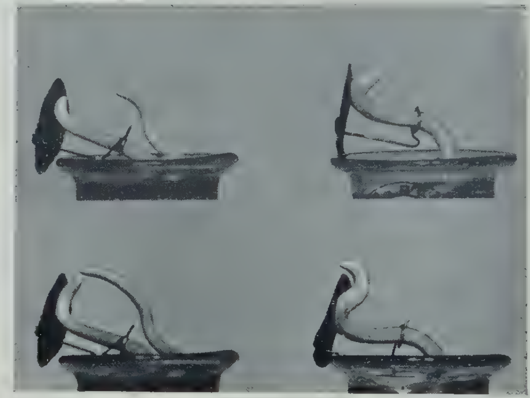

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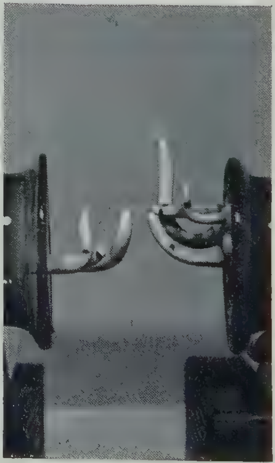

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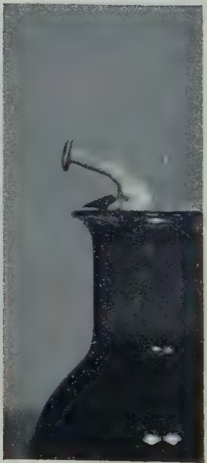

6

B. E. PLUNKETT

DESCRIPTION OF PLATE

FIG. 1. Formerly horizontal pileate-stipes (cf. stage figured in Fig. 6) which have curved downward phototropically as a result of a change of direction of illumination from one side to below.

FIG. 2. Positive phototropism of stipes placed in darkness after unilateral illumination at 1,500 f.c. for 240 secs. The ink marks were made at the apices at the time of treatment.

FIG. 3. Fruit-bodies which have developed with horizontal illumination from one side. The stipes show successive positive phototropic and negative geotropic curvatures. In the lower three fruit-bodies the central section of the stipe was originally horizontal but some sagging has occurred. This may be one of the causes of the lack of perfect verticality in the distal section of the stipe (see discussion).

FIG. 4. The effect of placing a disc of black paper 5 mm. from the apex of a phototropically orientated stipe between it and the light source. Upper two fruit-bodies: left, negative geotropism after growth towards the disc centre, right, the second phototropic curvature. Lower two fruit-bodies: the same two days later. The right-hand fruit-body now shows the 3rd phototropic curvature.

FIG. 5. Epileate stipes showing negative geotropic curvature after having been placed horizontally in darkness. The ink marks were made at the apices at the time of treatment.

FIG. 6. A photically orientated stipe upon which a young pileus has differentiated. The angle made by the stipe to the horizontal indicates the extent to which the light source was above the fruit-body. It is at this stage that negative geotropism of the stipe begins; although the light source remains fixed.

The Morphology of the Inflorescence in *Ranunculus bulbosus* L.

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With eight Figures in the Text

ABSTRACT

This paper records the form variation of 750 inflorescences of *Ranunculus bulbosus* L. collected randomly from each of two large colonies growing on permanent grassland.

Each inflorescence has a terminal flower, 1–4 bracts on the main axis and up to 8 flowers borne on cymes subtended by these bracts. Over 75 per cent. of each sample consists of inflorescences with 2 or 3 bracts on the main axis and 2–4 flowers. The number of flowers increases with the number of bracts on the main axis and evidence is given that the 4-bract 9-flowered inflorescence may be nearly the largest and most complex that can be produced under these conditions.

The distribution of flowers in the axillary cymes is such that the inflorescences tend to be radially symmetrical and pyramidal in form. This is so even though with increase in the number of bracts on the main axis the proportion of axillary flowers decreases in the lowest cyme and increases in the cyme above.

It is considered that the form and size of the inflorescence can be related to the vigour of the plant and to the mechanical and nutritional problems involved. A comparison of the various types of inflorescences found probably reflects the developmental sequence of flower production. It also indicates that there is competition between certain potential flower positions as the inflorescence develops.

INTRODUCTION

THE inflorescence of *R. bulbosus* L. is usually described as a few-flowered irregular cyme with a single terminal flower and a variable number of younger flowers borne on axillary cymes which are subtended by bracts on the main axis. The object of this investigation has been to study the inflorescence in the field to find out (*a*) the range of numbers of bracts and flowers borne on the inflorescences, (*b*) if any particular combination of bracts and flowers occurs more commonly than any other, and (*c*) whether the inflorescence is in fact irregular or whether certain morphological patterns can be recognized.

Two large colonies were selected for investigation, at Mickleham, Surrey (sample 1) and Englefield Green, Surrey (sample 2). They are about 10 miles apart and both are growing in dense grassland. Approximately 1,000 inflorescences were collected randomly from each site at the end of April and beginning of May 1959 and preserved in 70 per cent. alcohol. Most plants had only a single inflorescence borne terminally on the underground corm

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and surrounded at the base by a rosette of radical leaves. Some plants also had one or two smaller inflorescences in the axils of radical leaves. Most of the inflorescences collected were terminal although both samples included a few axillary ones. Great care was taken to ensure that the whole of each inflorescence was gathered. At the time of collection the inflorescences were developed to the stage where the oldest, terminal flower was open or just about to open. The flowering period is short and all the flowers were in an advanced stage of development so that there was no likelihood of further

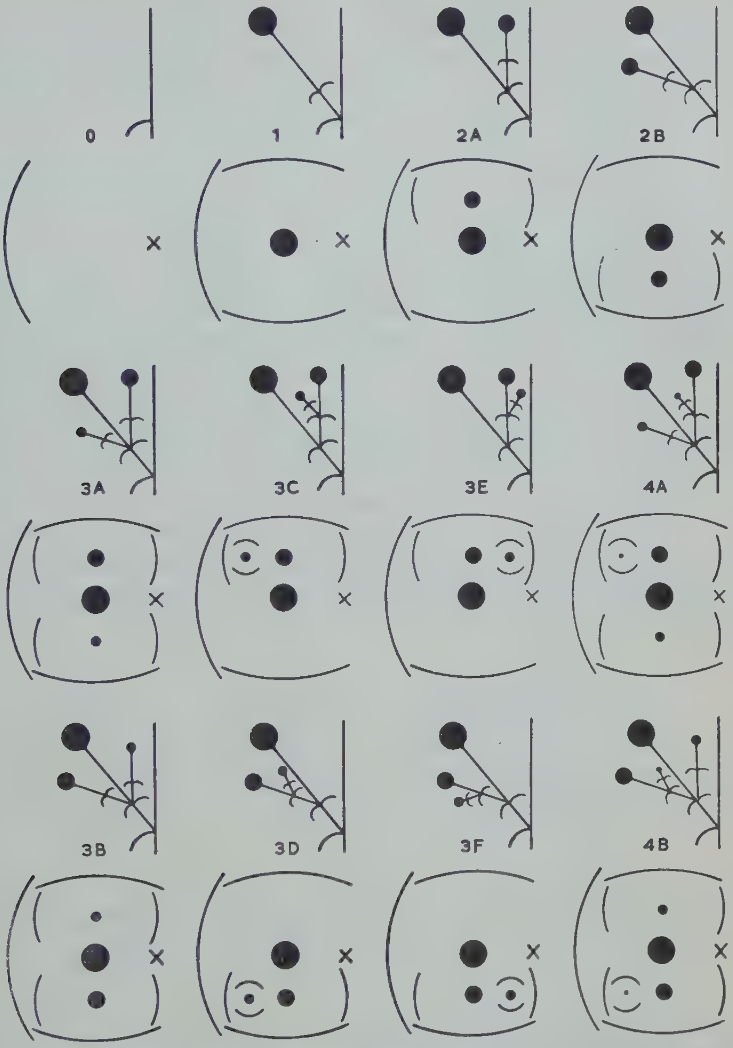

FIG. 1. Diagrammatic lateral views and plans of axillary cymes with 0–4 flowers as seen when held to the left of the main inflorescence axis. For further explanation see text.

flowers being formed had the inflorescences not been collected. Damaged inflorescences were rejected and finally 750 from each colony were used in the analysis.

The number of bracts on the main axis and the total number of flowers on each inflorescence were recorded. It was found that the axillary cymes bore 1–4 flowers only and the arrangement of these was also recorded. For a 1-flowered axillary cyme only one arrangement is possible, for a 2-flowered two arrangements, for a 3-flowered six arrangements, and for a 4-flowered cyme twenty possible arrangements. These are illustrated diagrammatically in Fig. 1 where only two of the possible 4-flowered cymes are shown because these are the only two which were at all frequent in the samples studied. To determine its arrangement each axillary cyme was held to the left of the main axis, in turn, and the inflorescence recorded as a series of digits and letters. For example, a 2A,1 inflorescence possessed 2 bracts and 4 flowers, an axillary cyme of 2 flowers subtended by the lower bract, a single flower subtended by the upper bract, and a terminal flower. A 3B,1,0 inflorescence had 3 bracts on the main axis and 5 flowers, a 3-flowered cyme in the axil of the lowest bract, a single flower subtended by the middle bract, no flowers in the axil of the upper bract, and the terminal flower. Where reference is made to the bracts they are numbered from below upwards, the lowest one being bract 1. The terminal flower of each axillary cyme is referred to as the 1st-order flower, those directly subtended by the two bracteoles on its pedicel as 2nd-order flowers, and any borne in the axils of the bracteoles of these are called 3rd-order flowers.

TOTAL NUMBERS OF FLOWERS AND BRACTS

The frequencies of inflorescences with different numbers of bracts and flowers are very similar in the two samples (Table 1). The inflorescences have 1–4 bracts on the main axis although the majority bear 2 or 3 bracts.

TABLE 1

Frequencies of Inflorescences with Different Numbers of Bracts and Flowers

Number of bracts	Sample	Number of flowers								
		1	2	3	4	5	6	7	8	9
1	1	5	3	1	0	0	0	0	0	0
	2	5	4	1	0	0	0	0	0	0
2	1	32	139	119	53	3	1	3	0	0
	2	14	131	88	57	6	1	0	0	0
3	1	19	61	77	152	26	14	5	0	0
	2	18	42	87	161	22	27	21	1	0
4	1	2	3	3	7	6	12	0	4	0
	2	1	1	13	10	9	6	13	10	1

On inflorescences with more than 1 bract the farther up the axis the bracts occur the less leaf-like they are in form. Where 3 or 4 bracts occur on the main axis bract 1 is often petiolate whereas those above are sessile and may have deeply cut laminas (Fig. 2).

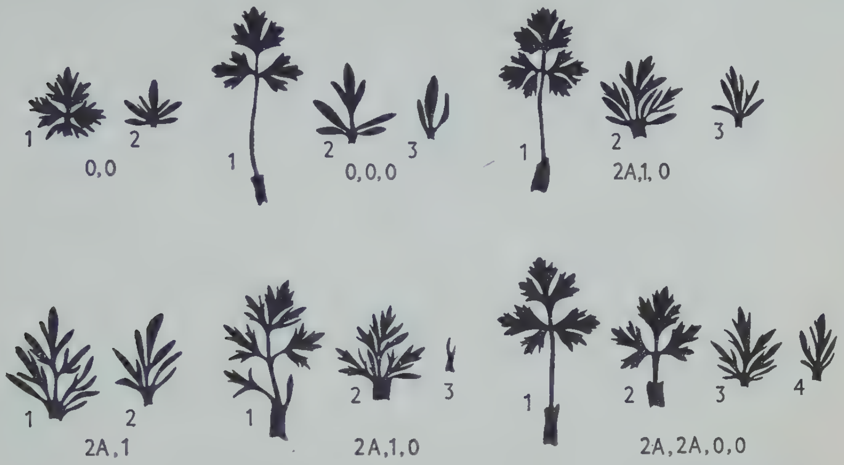

FIG. 2. Silhouettes of bracts borne on the main axis of selected inflorescences with 1–5 flowers. Bract 1 is the lowest. (× ⅓)

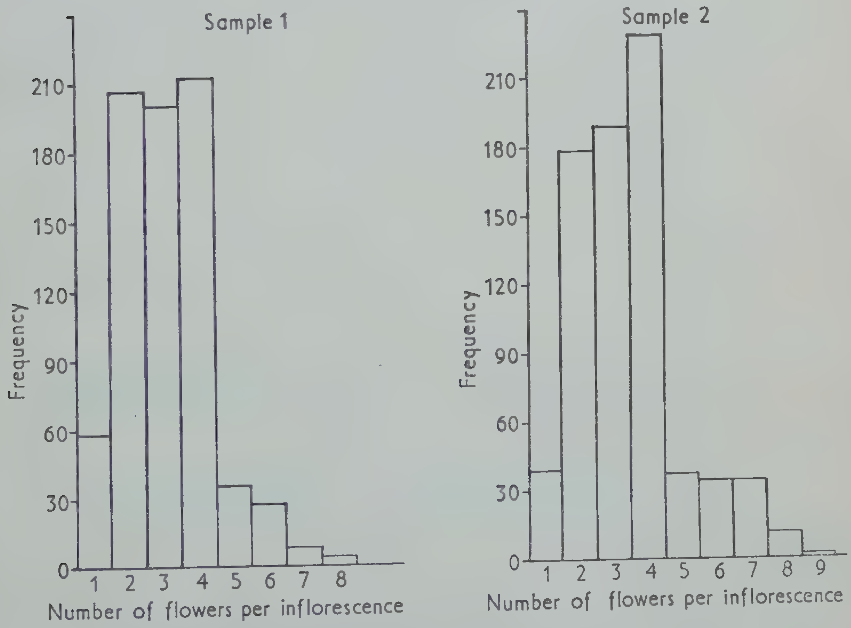

FIG. 3. Histograms of frequencies of inflorescences with different numbers of flowers.

In sample 1 the inflorescences have between 1 and 8 flowers and those of sample 2 have between 1 and 9 flowers. The frequency distributions of inflorescences with different numbers of flowers are very similar in the two samples (Fig. 3). Both samples include a number of 1-flowered inflorescences but most of the inflorescences have between 2 and 4 flowers each. In sample 1 there are almost equal numbers of 2-, 3- and 4-flowered inflorescences but in sample 2 there is a steady rise in numbers from the 2- to the 4-flowered inflorescences. Inflorescences with 5 or more flowers are relatively infrequent with a steady drop to the maximum of 8 flowers in sample 1 and 9 flowers in sample 2. The average number of flowers per inflorescence in sample 1 is 3·13 and in sample 2 3·46.

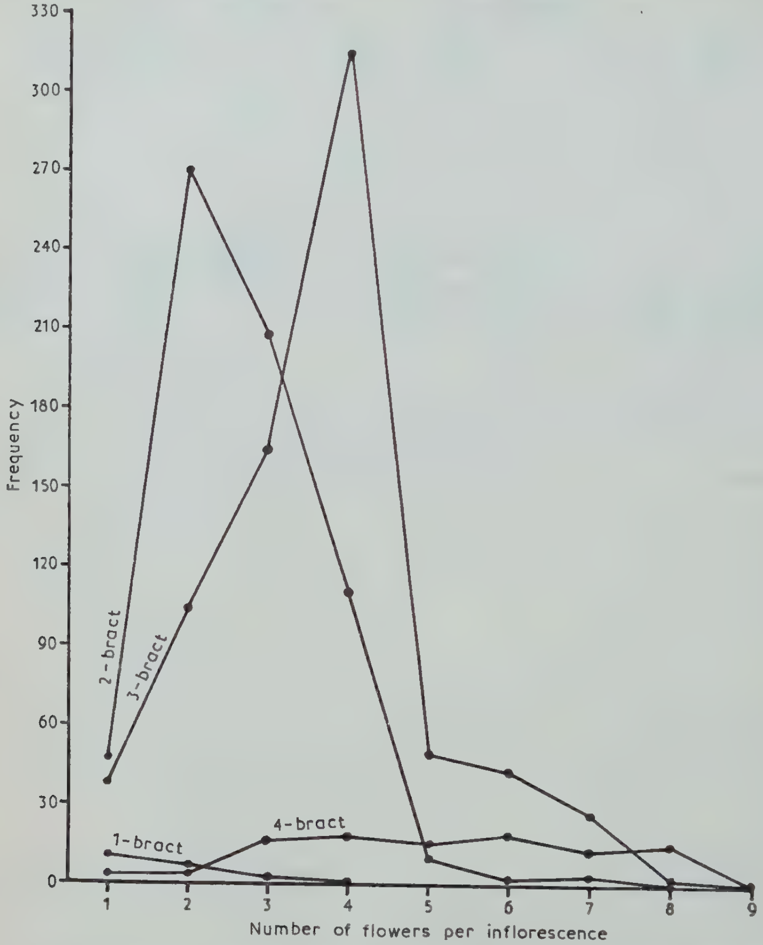

FIG. 4. Frequencies of inflorescences with different numbers of flowers, those with different numbers of bracts plotted separately. Both samples taken together.

Thus in sample 1 80·13 per cent. of the inflorescences possess 2 or 3 bracts and 2–4 flowers and in sample 2 75·47 per cent. of the inflorescences belong to these six classes. The relationship between numbers of flowers and numbers of bracts on the main axis may also be determined. The frequencies of inflorescences with different numbers of flowers and bracts are so similar in the two samples that they have been added in Fig. 4. The few 1-bract inflorescences are all small and none have more than 3 flowers. Conversely the few inflorescences with 4 bracts mostly possess between 3 and 8 flowers. Although there is no marked peak there are more 4-bract inflorescences with 6 than any other number of flowers. The 2-bract inflorescences have between 1 and 7 flowers with a maximum of 2-flowered ones, and very few with more than 5 flowers. On the other hand the 3-bract inflorescences have between 1 and 8 flowers with a maximum of 4-flowered types. There is a steep fall to the number of 5-flowered inflorescences and a further gradual fall to the very few inflorescences with 8 flowers.

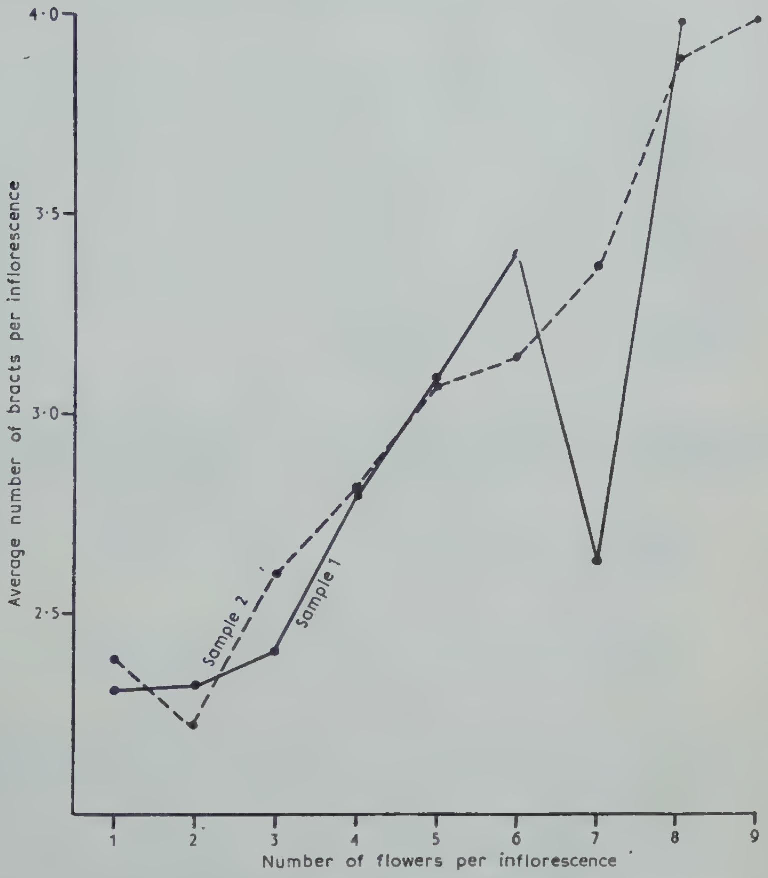

FIG. 5. Relationship between the total number of flowers on the inflorescence and the average number of bracts borne on the main axis.

The relationship between the number of flowers and bracts can be further illustrated by plotting the average number of bracts per inflorescence against number of flowers (Fig. 5). In sample 1 there is a slight rise and in sample 2 a slight fall in the average number of bracts between the 1- and 2-flowered inflorescences but from the 2-flowered to 6-flowered inflorescences there is a steep almost linear rise in both samples. There is a drop in the average number of bracts in the 7-flowered inflorescences in sample 1 but this is probably due to the fact that there are very few of these inflorescences in this sample. There is a further increase in the average number of bracts in the 8-flowered inflorescences in both samples.

NUMBERS OF FLOWERS IN AXILLARY CYMES

The data can be analysed further by considering the numbers of flowers borne on the axillary cymes subtended by the bracts on the main axis. This is most easily done by determining the total numbers of flowers borne at each position in inflorescences with different numbers of bracts (Table 2). Here again the two samples are very similar. Obviously in the few 1-bract inflorescences with more than one flower all the axillary flowers are borne in the cyme subtended by the only bract present on the main axis. In all inflorescences with two or more bracts there are more flowers in the lowest axillary cyme than in the one above and in inflorescences with 3 or 4 bracts there are fewer flowers in the third cyme than in the second.

TABLE 2

Total Number of Flowers in the Axillary Cymes at Different Levels on Inflorescences with Different Number of Bracts on the Main Axis

Position of bract on main axis	Sample 1 Number of bracts on main axis				Sample 2 Number of bracts on main axis			
	1	2	3	4	1	2	3	4
4th	—	—	—	0	—	—	—	0
3rd	—	—	2	16	—	—	2	27
2nd	—	68	304	58	—	73	365	102
1st	5	503	569	69	6	434	656	148

This is made clearer by expressing the frequencies as percentages of the total number of axillary flowers present on inflorescences with different numbers of bracts on the main axis. From Fig. 6 it can be seen that in sample 1 the lowest axillary cymes of the 2-bract inflorescences bear 88·1 per cent. of all the axillary flowers whereas in the 3-bract inflorescences they bear 65 per cent., and in the 4-bract inflorescences only 48·2 per cent. of the total number of axillary flowers. The corresponding figures for sample 2 are 85·6, 64·1, and 53·4 per cent. On the other hand there is an increase in the percentage of flowers borne on the second lowest axillary cyme with increase in number of bracts on the main axis. In sample 1 only 11·9 per cent. of the axillary flowers in the 2-bract inflorescences are borne on the cyme subtended by the second bract but in the 3-bract inflorescences 34·77 per cent. and in the 4-bract inflorescences 40·6 per cent. of the axillary flowers occur on this cyme. Again the corresponding figures for sample 2, 14·4, 35·7, and 36·8 per cent., are very similar. A similar trend is found in the axillary cymes subtended by the third bract in the inflorescences with 3 or 4 bracts.

In sample 1 the uppermost bracts of the 3-bract inflorescences subtend only 0·25 per cent. of the total number of axillary flowers whereas in the 4-bract inflorescences 11·2 per cent. of the axillary flowers are borne on the third cyme. For sample 2 the percentages are respectively 0·19 and 9·8. With the exception of one 4-bract, 7-flowered inflorescence in sample 2 the third bract never subtends more than a single flower and in none of the 4-bract inflorescences in either sample are any flowers found in the axil of the uppermost bract.

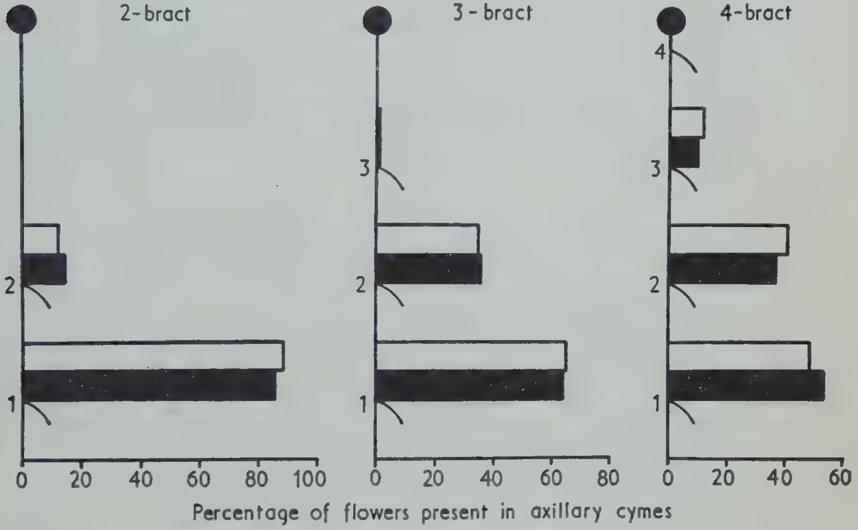

FIG. 6. The numbers of flowers borne on the various axillary cymes expressed as percentages of the total number of axillary flowers borne by each of the 2-, 3-, and 4-bract inflorescences. Sample 1 white; sample 2 black.

The relationship between the two lowest axillary cymes can be illustrated further by plotting the average number of flowers in the second axillary cyme against the number found in the first one. The two samples are taken together and the points for 2-bract, 3-bract, and 4-bract inflorescences are plotted separately (Fig. 7). Of the 2-bract inflorescences there are a few with no flowers in the axil of the lowest bract and one in the axil of the second and the average number of flowers in the second cyme where there is a single flower in the lowest is also very small. However, with further increase in the number of flowers in the lowest cyme there is a marked increase in the average number of flowers present in the cyme above but even at the maximum of 4 flowers in the lowest cyme there are never more than 2 in the second one and the average is 1·75. The corresponding averages of flowers in the second cyme of the 3-bract inflorescences all lie above those for the 2-bract inflorescences. With increase from 0 to 3 flowers in the lowest cyme there is a proportionately greater increase in the average number of flowers in the second cyme. In the 3-bract inflorescences with 4 flowers in the lowest cyme there are again never more than 2 flowers in the second cyme and the average, 1·83, lies only just above that for the 2-bract inflorescences. The points for the 4-bract inflorescences again lie above the corresponding ones for the 3-bract inflorescences.

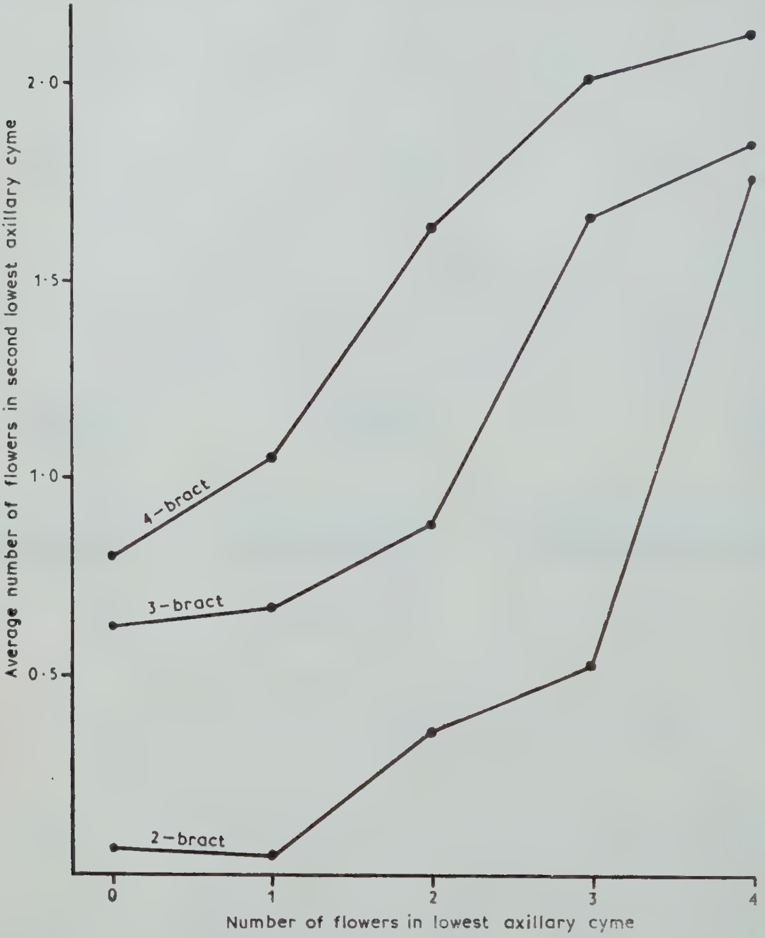

FIG. 7. Relationship between the number of flowers in the lowest axillary cyme and the average number of flowers in the second lowest axillary cymes in 2-, 3-, and 4-bract inflorescences.

It is noteworthy here that in inflorescences with one flower in the lowest cyme there is an average of 1·05 flowers in the cyme above it and with 2–4 flowers in the lowest cyme the average number of flowers in the second cyme is always more than half the number present in the lowest one. Thus, in comparing the three groups of inflorescences the difference between the numbers of flowers in the two lowest cymes decreases with increase in the number of bracts on the main axis. In the 3- and 4-bract inflorescences there is a decrease in the proportion of axillary flowers borne on the lowest cyme and an increase in the proportion borne on the second cyme in spite of the fact that some flowers also occur in the axil of the third bract above.

ARRANGEMENT OF FLOWERS IN THE AXILLARY CYMES

Most of the different arrangements of flowers in axillary cymes found in the two samples are illustrated diagrammatically in Fig. 1. There are only two possible types of 2-flowered cymes which, if held on the same side of the main inflorescence axis and viewed from above, are mirror-images of each other. The majority of the 2-flowered axillary cymes are subtended by the lowest bract on the main axis although in some of the larger inflorescences they occur in the axil of the second bract and one inflorescence was found in sample 2 with a 2-flowered cyme in the axil of the third bract. The frequencies of the two possible types of 2-flowered cymes 2A and 2B are given in Table 3.

TABLE 3

Frequencies of the 2A *and* 2B *Cymes Present in the Axils of Different Bracts on the Main Inflorescence Axis*

Number of bracts per inflorescence	Sample 1				Sample 2					
	Bract 1		Bract 2		Bract 1		Bract 2		Bract 3	
	2A	2B	2A	2B	2A	2B	2A	2B	2A	2B
1	1	0	—	—	0	1	—	—	—	—
2	74	69	2	1	69	65	1	2	0	0
3	90	106	18	15	112	89	32	29	0	0
4	12	7	14	9	3	12	16	2	0	1
Total	177	182	34	25	184	167	49	51	0	1

It is clear from Table 3 that the two types of 2-flowered cymes are equally likely to occur in the axil of the lowest bract on the main inflorescence axis and that this is in no way affected by the number of bracts borne above it. The same is also true of the 2-flowered cymes borne in the axil of the second bract. In all there are 211 type 2A cymes and 207 type 2B cymes on the sample 1 inflorescences and on the sample 2 inflorescences the numbers of the two types are 233 and 219 respectively. It appears therefore that the position of the younger flower on the 2-flowered cyme, i.e. whether it is borne in the axil of the left or right bracteole when the cyme is viewed from above, is entirely due to chance. Further, the morphology of the 2-flowered cyme is not affected by its position on the main inflorescence axis.

However, in those inflorescences with 5 or more flowers in which 2-flowered cymes occur in the axils of both the first and second bracts on the main axis there is evidence that the morphology of the second cyme is not completely independent of that of the one below it. Taking both samples together there are 37 inflorescences in which these two cymes are the same, i.e. 2A and 2A or 2B and 2B, whereas there are only 14 in which they are different, i.e. 2A and 2B or 2B and 2A. Although these frequencies are small they are highly significantly different ($P = < 0{\cdot}001$) from that expected if the two positions were entirely independent.

As illustrated in Fig. 1 there are six possible arrangements of 3 flowers in an axillary cyme. With the exception of one 8-flowered inflorescence and the single 9-flowered inflorescence in sample 2 all these 3-flowered cymes occur in the axil of the lowest bract on the main axis. The frequencies of the different types of 3-flowered cymes are given in Table 4.

TABLE 4

Frequencies of the Six Possible 3-flowered Axillary Cymes Subtended by the Lowest Bract on the Inflorescence Axis

	Type of 3-flowered cyme					
	3A	3B	3C	3D	3E	3F
Sample 1	9	14	5	5	0	1
Sample 2	19	26	10	15	1	1
Total	28	40	15	20	1	2

Both the 3A and 3B cymes are dichasial with a single second-order flower borne in the axil of each of the two opposite bracteoles on the pedicel of the first-order flower. One second order flower is older than the other and the 3A and 3B cymes differ only in that they are mirror-images of each other when held on the same side of the axis and viewed from above. Taking both samples together there are nearly twice as many cymes of these two types than there are of the other four types 3C to 3F. The frequencies of the two dichasial types do not differ significantly from those expected by chance.

Types 3C to 3F are monochasial cymes with a third order flower in the axil of one of the bracteoles on the pedicel of the single second-order flower. The 3C and 3D cymes are mirror-images of each other and although there are far fewer of these than of the dichasial 3-flowered cymes the frequencies of these two types are again not significantly different from a chance distribution. The 3E and 3F cymes are mirror-images of each other and also of the 3C and 3D cymes respectively, differing from the latter in that the third-order flower is borne in the axil of the bracteole on the pedicel of the second-order flower which is towards, and not away from, the main inflorescence axis (Fig. 1). However, these types are very infrequent, only one such axillary cyme being found in sample 1 and two in sample 2. Types 3A, 3C, and 3E are similar in that the second oldest flower is on the same side of the cyme when viewed from above whereas types 3B, 3D, and 3F bear this flower on the opposite side. There are 44 cymes in the first group and 62 in the second but here again the frequencies do not differ significantly from a chance distribution.

A further point may be investigated in the 59 inflorescences from both samples in which there is a 3-flowered cyme in the axil of the lowest bract and a 2-flowered cyme in the axil of the bract immediately above (Table 5).

Although the numbers are small there is nothing to suggest that the type of 2-flowered cyme borne in the axil of the second bract is not completely independent of the type of dichasial cyme (3A or 3B) borne in the axil of the bract

TABLE 5

Frequencies of Inflorescences with Different Types of 3-flowered Cymes in the Axil of the Lowest Bract and the Two Types of 2-flowered Cymes in the Axil of the Second Bract

Type of 2-flowered cyme in the axil of the second bract	Type of 3-flowered cyme in the axil of the lowest bract			
	3A	3B	3C	3D
2A	14	10	5	3
2B	11	11	1	4

immediately below it. The figures for the other combinations in Table 5 are too small to test statistically. However, it is worth noting that of 13 inflorescences 9 belong to the 3C, 2A and 3D, 2B groups, i.e. where the second-order flower is on the same side of each cyme when viewed from above, but only 4 to the 3C, 2B and 3D, 2A groups where the second order flowers are on different sides in the two cymes when viewed from above.

Taking both samples together there are 46 4-flowered cymes which all occur in the axil of the lowest bract on inflorescences with six or more flowers and 2–4 bracts. Of these 20 are type 4A and 22 type 4B (Fig. 1). These both have 2 second-order flowers and differ only in the position of the single third-order flower. They are mirror-images of each other and the numbers clearly indicate that their occurrence is due to chance alone. Sample 2 includes one type 4C cyme and three type 4D cymes. These differ from types 4A and 4B in that the single third-order flower is borne towards the main inflorescence axis as in the 3E and 3F cymes, and like these 3-flowered cymes they are very rare in these samples. The twelve other possible 4-flowered cymes are almost or entirely monochasial but such types do not occur in either sample.

DISCUSSION

The data presented above show that the samples of inflorescences drawn from two colonies of *R. bulbosus* plants are very alike. It should also be mentioned that a further sample collected from site 2 in 1960 was very similar to those collected in 1959. Therefore it may be considered that these samples give a reasonably true picture of the inflorescences produced in this type of habitat.

Salisbury (1942) demonstrated the effect of competition on the production of fruiting heads per plant in *R. bulbosus*. Without competition 17 plants had between 4 and 93 fruiting heads, and hence at least this number of flowers, with an average of 21·8. Clearly most of these plants must each have had either a larger and more complex single inflorescence than those described above or several separate flowering stems arising from the corm. On the other hand, this data on the number of fertile carpels produced by plants growing under competition indicates that very few flowers were borne on each plant. Salisbury pointed out that it is the reproductive capacity of a species under the conditions of competition in the habitat which it normally frequents that is usually of most biological importance. This argument is equally applicable to a study of the morphology of the inflorescence. Under the field conditions of intense competition the lower part of the range of inflorescence morphology can be investigated from the single-flowered inflorescence upwards. However, it is only under more favourable conditions that the upper part of the range of inflorescence morphology can be investigated.

A number of varieties of this species have been described but according to Drabble (1932) *R. bulbosus* L. var. *bulbifer* Jord., the most abundant one and the one to which my samples belong, has only one or two flowering stems arising at the top of the corm. Harper (1957) states that plants with large corms may bear up to 8 flowering stems but on neutral permanent grassland there is normally only one flowering stem. Nearly all the plants from which my samples were taken also had only a single inflorescence. Therefore under these field conditions it appears that up to a certain limit only a single inflorescence is produced and that its complexity and the number of flowers it bears are indicative of the size and vigour of the plant and of the amount of food material available. Beyond that limit more flowering stems are produced on the corm.

The main inflorescence axis bears 1–4 bracts. The lowest bract where 3 or 4 are present often has a long petiole and is structurally indistinguishable from a normal foliage leaf. As the inflorescence axis is terminal and a continuation of the axis of the corm it may be argued that this lowest bract is a vegetative leaf which, due to the elongation of the internode below it, is not radical. However, in most specimens an axillary cyme of one or more flowers is subtended by the lowest bract whether it is leaf-like or not. Therefore there is no doubt that this is part of the single, terminal inflorescence.

The inflorescences have between 1 and 9 flowers with an average of 3·3 overall. Approximately 75 per cent. of the inflorescences in each sample bear 2–4 flowers and there are relatively few with more than 5 flowers. As only inflorescences were collected and the size of the sample was small compared with the size of the colony two classes of plants were excluded. These were (*a*) the many small plants without inflorescences at all at the time of collection and (*b*) the few large plants bearing more than 9 flowers. Even if some of the small plants produced flowers later on in the season the effect of the first class would be to lower still further the average number of flowers produced per plant whereas the second class, due to its scarcity, would be unlikely to raise the average significantly.

There is generally an increase in the total number of flowers with increase in the number of bracts on the main axis (Figs. 4, 5). The 1-bract inflorescences have 1–3 flowers with the maximum at 1 flower, the 2-bract inflorescences have 1–7 flowers with the maximum at 2 flowers, the 3-bract inflorescences have 1–8 flowers with the maximum at 4 flowers and the 4-bract inflorescences have 1–9 flowers with the maximum at 6 flowers. This picture is limited by the few 1- and 4-bract inflorescences included and by the absence of any inflorescences with more than 4 bracts on the main axis and more than

9 flowers. It would be interesting to know whether, under more favourable conditions, the inflorescence axis could bear more bracts and hence more axillary cymes or whether 4 bracts is the upper 'hereditary' limit. If in fact an inflorescence axis never bears more than 4 bracts then more flowers can only be produced by the 4 cymes in their axils. This may impose an upper limit on the number of flowers borne by one inflorescence due to either mechanical or nutritional limitations of a single main axis. As mentioned previously both samples included a few specimens from plants with more than one flowering stem and it may be that the 4-bract 9-flowered inflorescence is very near the upper limit of complexity that can be produced under these conditions.

Where only a single bract is borne on the main inflorescence axis all the axillary flowers must occur on the one cyme subtended by that bract. Such inflorescences are rare and there are never more than 2 flowers present on the axillary cyme. Far commoner are the 2-bract inflorescences where the flowers are distributed in 2 axillary cymes. In most of these there are more flowers in the lowest axillary cyme than in the one above. Particularly common are the 1,0, 2,0, and 2,1 types. The largest are the few 4,2 inflorescences of sample 1. With 3 bracts on the main axis the number of possible arrangements of axillary flowers is greatly increased. The commonest is the 2,1,0 type and the largest is an 8-flowered 4,2,1 inflorescence in sample 2. Relatively few 4-bract inflorescences occur in the samples but the commonest of these are the 3,2,1,0 and 4,2,1,0 types and the one 9-flowered inflorescence of sample 2 is a 4,3,1,0 type. Thus, although many types of inflorescences occur in these samples, varying in number of bracts and number and distribution of flowers, the majority have a pyramidal morphology with a single terminal flower and increasing number of flowers in the axillary cymes from the top to the bottom of the main axis. This is true of most of the inflorescences in spite of the fact that with increase in the number of bracts on the main axis the proportion of axillary flowers borne in the lowest cyme decreases.

Axillary cymes with two flowers are common and whether they are of the 2A or 2B types appears to be a matter of chance. However, on inflorescences where there are 2-flowered cymes subtended by each of the two lowest bracts they tend to be of the same kind. The two lowest bracts are more or less on opposite sides of the main axis so that if they subtend 2-flowered cymes of the same kind the second-order flowers of these will project in opposite directions and the whole inflorescence will tend to appear symmetrical when viewed from above.

A majority of the 3-flowered cymes are dichasial and therefore symmetrical. Most of the monochasial ones have the single third-order flower directed away from the main axis thus giving a more spreading form to the inflorescence. Where a monochasial 3-flowered cyme occurs in the axil of the lowest bract together with a 2-flowered cyme in the axil of the second bract the second-order flowers tend to project in opposite directions with respect to the main axis thus again giving symmetry to the whole inflorescence. Most of the 4-flowered cymes are like the dichasial 3-flowered forms plus a single third-order flower and none of the possible monochasial asymmetrical types are found.

Thus there is considerable evidence indicating that the distribution of the flowers is such that the whole inflorescence tends to be pyramidal and symmetrical. Even though the number of flowers is small this arrangement enhances the mechanical stability of the inflorescence. If there were several flowers in the uppermost axillary cyme and few or none in the lowest one, or most of the flowers were borne to one side of the main axis, the whole inflorescence would not be so stable. It may be argued that in such an inflorescence with few flowers mechanical support is not very important. On the other hand the axes of the inflorescence are slender, the flowers are large and the fruiting heads quite heavy. Further, each pedicel has two bracteoles and potentially a flower could develop in the axil of each so theoretically an inflorescence could bear a large number of flowers. As pointed out earlier the 9-flowered inflorescence may be nearly the largest and most complex that the plant can possibly produce and this could be due as much to mechanical as to nutritional limitations.

So far the morphology of the inflorescence has been considered in relation to its mechanical stability and the vigour of the plant but its development may also be briefly discussed. Although development has not actually been followed in this investigation certain points can be made in the light of the data given above. Presumably the main axis itself develops basifugally from the meristem at the apex of the corm, forming first the lateral bracts and then the terminal flower. The latter is the first flower to open and is morphologically the oldest so that it is almost certainly the first flower to be initiated on the young inflorescence. Once the main axis with its terminal flower has been laid down further development can only take place from the buds in the axils of its bracts. In very small plants these buds fail to develop at all and a 1-flowered inflorescence results (Fig. 8).

In 1-bract inflorescences any further flowers must be formed on the cyme subtended by the only bract present. If one further flower is formed on a 2-bract inflorescence it will usually be in the axil of the lowest bract on the main axis. When a third is formed it may be either a first-order flower in the axil of the second bract or a second-order flower on the lowest cyme. Hence it would appear that there is some competition between these two positions although the lower one predominates as there are far more 2,0 than 1,1 inflorescences in the samples. With the formation of a fourth flower in the 2-bract inflorescences both the 1,1 and 2,0 types can give rise to the very common 2,1 arrangement. The only frequent 5-flowered 2-bract inflorescence has the 3,1 form so that here again the lowest cyme predominates over the one above.

A somewhat similar sequence may be construed from the very numerous 3-bract inflorescences. There are almost equal numbers of 1,0,0 and 0,1,0 2-flowered inflorescences which indicates that here there is competition between the two lower axillary buds with an almost equal chance of the single

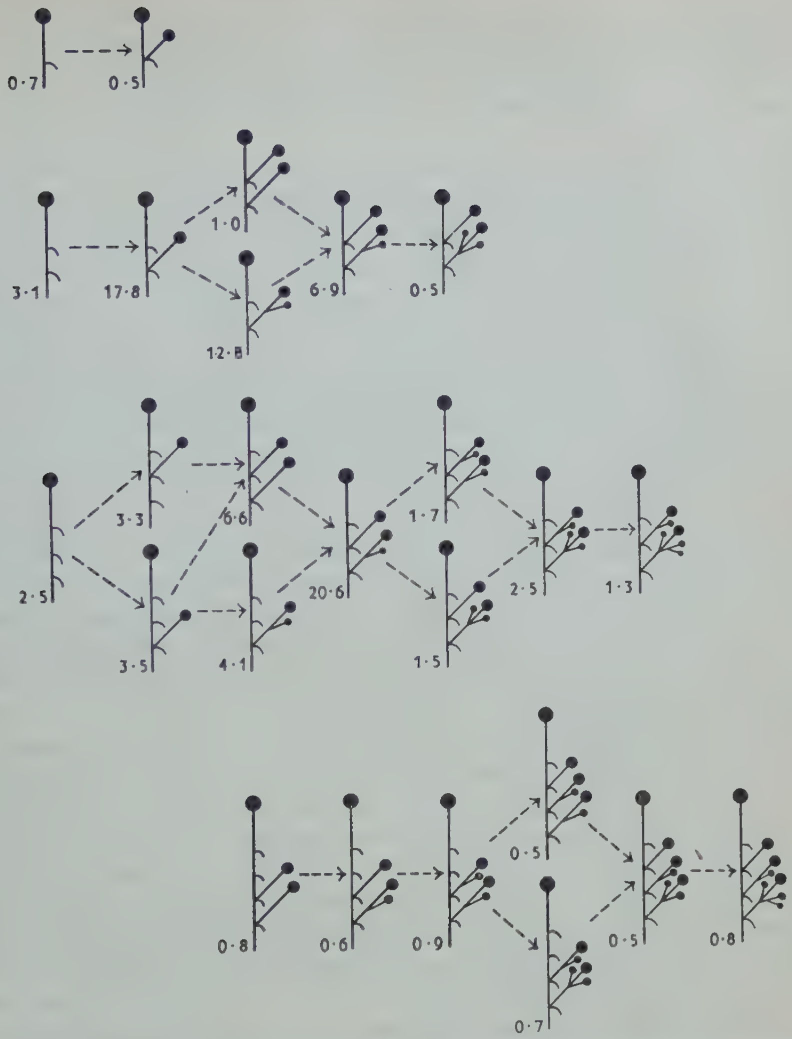

FIG. 8. Diagrams of the most frequent kinds of inflorescences found in the samples. The numbers represent the percentage occurrence of each in the total of 1,500 inflorescences examined. For further explanation see text.

lateral flower appearing in either position. Both forms can give rise to the 1,1,0 type which is the commonest 3-flowered, 3-bract inflorescence. Nearly as common is the 2,0,0 inflorescence which can only develop from the 1,1,0 form. As in the 2-bract inflorescences both these common 3-flowered forms can give rise to a single type of 4-flowered inflorescence. This is the extremely common 2,1,0 type which comprises over 20 per cent. of all the inflorescences included in the two samples taken together. If a fifth flower is formed it is equally likely to occur in a second-order position on either of the two lower cymes and again there are almost equal numbers of the 2,2,0 and 3,1,0 forms.

Again both of these can give rise to the 6-flowered 3,2,0 inflorescence. The only common 7-flowered 3-bract inflorescence is the 4,2,0 form so that here one of the third-order positions of the lowest cyme predominates over both the vacant second-order position on the second cyme and the axil of the uppermost bract.

There are not a great many 4-bract inflorescences but again a sequence of flower production can be suggested. The only frequent 3- to 5-flowered inflorescences are the 1,1,0,0, 2,1,0,0, and 2,2,0,0 forms. However, there are two 6-flowered types, the 2,2,1,0 and 3,2,1,0 inflorescences which are equally common. On the assumption that they are both derived from the 2,2,0,0 form this indicates that there is competition between the vacant second-order position on the lowest cyme and the axil of the third bract. Both these types can give rise to the only common 7-flowered 3,2,1,0 inflorescence.

A further point may be made by comparing the 3-flowered and also the 5-flowered inflorescences with 2 to 4 bracts. Of the 3-flowered inflorescences the 2,0 far outnumber the 1,1 types but there are fewer 2,0,0 than 1,1,0 types and in the 4-bract inflorescences only the 1,1,0,0 form occurs at all frequently. Similarly in the 5-flowered inflorescences the 3,1 type is the only frequent 2-bract inflorescence, the 3,1,0 and 2,2,0 forms are of equal occurrence in the 3-bract inflorescences but it is the 2,2,0,0 form which is the only common 4-bract type. This indicates that with increase in the number of bracts on the main axis from 2 to 4 the dominance of the lowest cyme over the one above steadily decreases.

It should be emphasized that this interpretation of developmental sequence is based entirely on the comparative morphology of the inflorescences but it seems a reasonable one in the light of the frequency of occurrence of the commoner types. It may also be that the morphology of the inflorescence is to some extent affected by spacial factors as it develops and that its pyramidal and symmetrical features are the result of efficient packing in the apical bud.

Finally it may be stated that in these inflorescences of *R. bulbosus* collected in the field there is a narrow range of number of bracts and flowers, some combinations of bracts and flowers are particularly common, and that although there is considerable variability certain morphological patterns can be recognized.

ACKNOWLEDGEMENTS

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The Crystals in the Ovaries of certain Compositae

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With twelve Figures in the Text and two Plates

ABSTRACT

The inner tissues of the ovary wall in many Compositae contain crystals of calcium oxalate. These are of many different forms, some of which are of very restricted taxonomic distribution. These crystals are organized inclusions of the cell, many of them having a complex internal structure which is not explicable in terms of a homogeneous crystal lattice.

IN the ovary wall of many Compositae there is a layer of parenchyma, often more or less deliquescent, which contains numerous microscopic crystals. Examination of these bodies has revealed some remarkable features of which I can find no previous record. The available chemical evidence suggests that the crystals consist of calcium oxalate, but their morphology is quite incompatible with the views which most botanists seem to hold regarding the structure of calcium oxalate crystals occurring in plants.

The general impression is that calcium oxalate crystallizes in a cell just as it would elsewhere. Most writers assume each crystal to be a homogeneous mass, formed under the influence of purely physical laws, and displaying no sign of active protoplasmic interference. Although several crystal forms are recognized in the literature their taxonomic distribution has encouraged the belief that a given type of crystallization is an accidental by-product of some unspecified physiological condition, not a thing rigidly determined by a particular genotype.

In two respects this picture is modified by the investigation now to be described. In the first place, some of the crystals discovered display a high degree of structural organization of a distinctively biological kind. Secondly, some crystal forms are of such limited taxonomic range that it is hardly possible to deny the existence of a close genetical control.

MATERIALS AND METHODS

The crystalliferous tissue lies in the inner part of the ovary wall. An adequate specimen can usually be obtained easily by scraping or peeling the inner face of the wall at about the time of anthesis. The material can then be dried upon a cover-glass and mounted in balsam or subjected to chemical treatment as required. In many species crystals can be recovered without difficulty from herbarium specimens.

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It is neither necessary nor desirable to cut sections for the routine examination of these crystals. Because of the curvature of the crystalliferous layer a section can show only a small part of it, and unless the crystals are small a high proportion of them are likely to be broken by the knife. In most cases the tissue is so delicate as to offer no obstacle to observation of the crystals when dissected out; in the few instances where the parenchyma is unusually dense it is best to couple dissection with a bulk-clearing technique.

Almost every preparation contains a proportion of fractured specimens and the larger crystals are easily broken by rough handling. Excessive heating also results in breakages. It is not known whether crystals are ever broken naturally in the living material. As, however, some species have long slender crystals densely packed in a tissue which undergoes both elongation and flexure, the possibility of natural breakages cannot be excluded.

Although a typical crystalliferous layer is known to exist in some other tribes, the present investigation is concerned only with members of the Cynareae. The crystals of four species, selected to show obvious structural differences, have been examined in some detail, and enough has been seen of other forms to permit of some preliminary remarks on the taxonomic issues.

THE APPLICATION OF ACIDS

Calcium oxalate is completely insoluble in acetic acid, but soluble in hydrochloric acid. In sulphuric acid it is said by most authorities to be converted into calcium sulphate, which is itself insoluble in the acid and which retains approximately the outline of the original oxalate crystal.

I have not carried out all these reactions with the crystals of every species examined. In particular it seemed pointless, after a number of trials, to repeat the application of acetic acid. I believe that any of the crystals described could be exposed for an indefinite period to acetic acid of any concentration without visible effect.

The reactions with hydrochloric acid and sulphuric acid have not yielded any result inconsistent with the view that the crystals are composed basically of calcium oxalate, but it has become apparent that these reactions are more complex than has commonly been supposed, and it has been necessary to devise means for controlling them so as to permit detailed observation.

With hydrochloric acid it is possible to take advantage of the volatility of the reagent. A glass cell-ring is fastened to a slide and a few drops of concentrated hydrochloric acid placed in the bottom, keeping the upper face of the cell dry. A cover-glass bearing the crystals is placed over the cell; no grease is needed here. Then by warming the apparatus on the hand a little acid can be distilled over on to the specimens, the effect being observed intermittently through the microscope. At any desired point the reaction can be stopped by removing the cover and either warming it to drive off the acid or mounting it on a slide with a drop of alkali. In studying the earlier stages of the reaction it is quite feasible to evaporate the acid and make permanent preparations with balsam. With the later stages this generally cannot be done and it is then more satisfactory to neutralize the acid and to content oneself with a temporary mount.

While the crystals are in the reaction-cell the acid is attacking them from one side but they are under observation from the other; if the crystals are on a cover-glass this relationship will be maintained in any subsequent mount. For really critical work on the very first stages of the reaction this situation is not always acceptable. It may be remedied by working with crystals on a slide, or alternatively by making a sandwich mount with two covers.

The reaction with sulphuric acid is difficult to control and the effects produced change surprisingly as the conditions are varied. It has been no part of my plan to attempt a detailed study of the influence of acid concentration, &c., but it seems desirable to place on record the three distinct types of reaction which I have been able to bring about by varying mechanically the freedom of access of the acid to the crystal specimens.

To secure a very rapid reaction a cell is prepared and is loaded with a drop or two of concentrated sulphuric acid. A cover-glass bearing crystals is then attached firmly to the top of the ring, and the whole apparatus is placed, without tilting, in the mechanical stage of a microscope. When a suitable group of crystals has been located the slide is removed from the stage, momentarily inverted, and swiftly returned so that the selected group of crystals comes under observation again. In these circumstances the usual statements about the replacement of oxalate by sulphate are quite untrue. I have observed the reaction many times, reducing the delay between the arrival of the acid and the first observation to less than a second, and I have never been able to detect the formation of new crystalline material. All that can be seen is a swift diminution of the original crystal; in a few seconds it vanishes without trace. Hydrochloric acid often has a selective effect upon particular parts of a crystal, but in rapid reactions with sulphuric acid nothing of the kind appears; material is removed evenly from all parts of the outer surface.

To bring about a slow reaction, crystals are arranged upon a cover-glass, longitudinal slices of any convenient parenchymatous tissue are added, and the whole assembly is dried. If now this cover is inverted upon a drop of concentrated acid upon a slide, the acid has to percolate through a layer of dried plant tissue before it can attack the crystals lying against the glass. In these conditions each crystal slowly fades away, being replaced by an aster of delicate needles extending outwards in all directions to a distance several times the radius of the original crystal. These needles are essentially temporary, and their form is soon altered by repeated solution and recrystallization. Eventually large reticulate or dendritic aggregations are formed, extending for long distances through the preparation and incorporating material derived from large numbers of the original crystals, the positions of which can no longer be recognized.

When, without any special precautions, a sample of crystalliferous tissue is mounted in sulphuric acid, conditions will vary in different parts of the preparation. Where the degree of exposure of the crystals to the acid is intermediate between the two extremes just described one can often observe the conversion of the original material into a granular mass, obviously crystalline in character but not geometrically regular in outline. These granular bodies are fairly permanent, and will lie in the acid without major alteration at least for several hours. Sometimes little brush-like groups of needle-crystals arise on their outer surfaces as a secondary incrustation.

CHARACTERISTIC CRYSTAL TYPES

Centaurea scabiosa

The normal crystals of this species are remarkably uniform in size, being generally about $12 \times 8 \times 6\mu$. A face view (Plate 1, Fig. 1) has smoothly curved sides, which do not give the slightest indication of angles or facets. The ends may be either truncate, the crystal then terminating in a line which is perfectly straight and accurately transverse, or pointed, in which case the curved sides meet in a rather blunt rounded apex. The two ends of the same crystal are often very dissimilar, a common type being boat-shaped, with one end pointed and the other truncate. Even when both ends are truncate they are often very unequally so. No statistical study has been undertaken, but certainly in my material truncate ends predominated, crystals with both ends pointed being noticeably uncommon. In side view (Plate 1, Fig. 2) a crystal always appears as a perfect rectangle, from which it follows that the faces must be flat, parallel, of identical form, and exactly superposed.

A small proportion of crystals, in my material always much less than 1 per cent., are very much wider than is normal. These specimens display the usual degree of curvature of the sides (Plate 1, Fig. 3) but have enormously long, perfectly straight ends. It is surprising that the shape of the sides is so little affected by so greatly increasing the distance between them.

Every normal crystal possesses a characteristic marking which will be called the *central body*. This has a different refractive index from the surrounding substance, and appears light or dark according to the place of focus. When a crystal has two similar ends the central body is just half-way between them. When the ends are unequal the central body is a little nearer to the more truncate end of the two. If a crystal were cut transversely through its central body the two parts would often be very different in shape, but it is probable that they would always be approximately equal in volume. In the exceptionally wide crystals the presence of a central body has not been satisfactorily demonstrated; this may only mean that the central body material, being distributed across a greater width, is so attenuated as to escape observation. The central body has no distinct outline, merging gradually into the general substance of the crystal on all sides. It is totally immersed in the crystal mass, and may be regarded provisionally as a rather thick lens or disk placed transversely across the median longitudinal axis of the crystal.

The crystalline material, including the central body, is completely soluble in hydrochloric acid. After its removal a thin membranous bag is left which retains the original outline of the crystal and which was presumably present as an outer covering on the intact specimen. This will be called the *envelope*. It appears quite uniform and featureless, and is much too thin to permit accurate visual assessment of its thickness. The envelope is destroyed by sulphuric acid.

The initial attack of hydrochloric acid upon these crystals is highly selective. The acid begins by drilling a number of fine holes or canals (Plate 1, Figs. 4 and 5). These drillings enter the crystal through the sides, in a transverse direction and parallel to the faces. Although a crystal may ultimately be drilled very extensively the first attack seems to be concentrated always upon a very small part of the side surface. Each crystal may be said to possess two target areas, one on each side, equidistant between the two faces, and lying in the transverse plane which passes through the central body. A side view of a crystal in a fairly early stage of erosion by hydrochloric acid shows a scatter-pattern, with a concentration of holes over the central body. I suspect, though I have not been able to prove, that the examination of specimens in the earliest possible stage of solution would show always a single hole on each side, and that accurately upon the target position. As time goes on the appearance of new holes is influenced less and less by the position of the central body and eventually the whole side area of a crystal becomes riddled from end to end, though always with some concentration of activity upon the mid-line of the side, that is to say upon the line equidistant between the faces. Drilling through the faces themselves seems to be very rare, and has been observed only immediately over the central body, where a single hole occasionally appears in the transverse plane, but at right angles to the faces and so also at right angles to the holes in the sides.

The principal action of the acid upon the faces is to etch upon them a pattern of grooves (Plate 1, Fig. 6). Although these grooves are often connected in a reticulate manner, they run predominantly in the transverse direction, and they are continuous with grooves which pass down the sides from one face to the other. The crystals consequently assume an appearance for which there are close parallels in the erosion of rock formations. Particularly when crystals are viewed in optical section the impression of a regular transverse stratification is very strong (Plate 1, Fig. 7). There can be little doubt that the acid in this way reveals a genuine property of the crystal, for the conditions may be varied very considerably without affecting the appearances produced. The combined thickness of the two layers of which each lamination must be supposed to consist, one more resistant to the acid than the other, is always a little less than one micron.

Soon after the stratification has become conspicuous, the hydrochloric acid takes a clean bite through the centre of the crystal (Plate 1, Fig. 8). Then for a considerable time there may remain two crystalline bodies, slowly diminishing in size, and sometimes in the final stages undergoing some further fragmentation, enclosed within the envelope (Plate 1, Fig. 9). This division of the crystal into two is highly characteristic. Exceptional cases occur in which there is more than one transverse division, so that the crystal is cut into three or more parallel plates, but these instances are very uncommon.

All the features which can be demonstrated in these crystals by the use of hydrochloric acid are essentially transverse, and one might therefore anticipate that other agents would act in a similar manner. The facts are quite otherwise.

Significant changes can be caused by heating the crystals in a flame. They are relatively resistant to this treatment, and will bear an exposure which would completely shatter the crystals of some of the other species examined. Upon heating the central body disappears, completely and, so far as is known, irreversibly. Upon the side of the crystal a line appears which is parallel to the faces and equidistant between them (Plate 1, Fig. 10). In view of the position in other species it can hardly be doubted that this line is a *joint plane*, the crystal having really a paired structure, with two similar components joined together face to face. But the most surprising effect of heat is to produce upon the exposed faces a system of parallel longitudinal striations (Plate, 1 Fig. 11). These appear to be cracks or fissures.

Sulphuric acid, when its access to the specimen is restricted to a moderate extent, also produces a characteristic and perfectly reproducible effect (Plate 1, Fig. 12). Each component is converted into a granular mass, presumably of calcium sulphate, and undergoes at the same time an appreciable change in dimensions. The depth is disproportionately increased, so much so that a crystal in this condition will no longer lie upon its face (indeed flat faces no longer exist) and falls over upon its side. Between the two components there appears to be a layer of some kind of cement, which even in sulphuric acid remains non-granular and quite rigid. The cement probably does not extend to the ends of the crystal, because at these points the two components quite constantly and characteristically curve slightly away from each other as shown.

Silybum marianum

The crystals of this species vary enormously in size. The variation appears to be bimodal, with one class of large crystals, about 45μ long on the average, and another of small crystals, averaging about 10μ in length. Intermediates occur, but are relatively uncommon. The smaller crystals are difficult subjects, and their basic structure, so far as it can readily be ascertained, does not significantly differ from that of the larger ones. Observations have therefore been made mostly upon the larger type. Crystals of different sizes are not randomly distributed in the ovary. Small crystals occur only in special patches of crystalliferous tissue which seem to produce only crystals quite near to the minimum size. These little crystals then occur in dense swarms and must very greatly outnumber the cells which produce them. The larger crystals are much more generally distributed but less crowded, and it seems likely that a cell can normally produce only one or two of them.

In face view a crystal presents the appearance of an elongated hexagon with pointed ends (Plate 1, Fig. 13). A specimen of the largest size is about

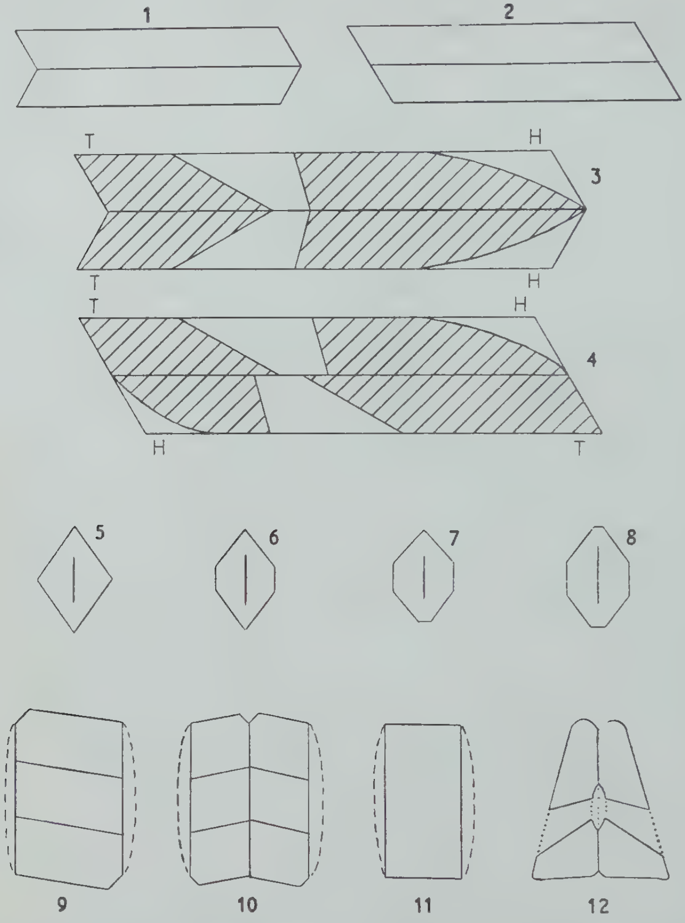
$60 \times 12 \times 10\mu$. In crystals of intermediate size similar proportions are maintained, but in the smallest sizes, although the ratio of length to breadth is not much altered, the depth seems always to be disproportionately reduced. Crystals near the minimum size are mere plates, so thin that examination from the side yields no information at all. The difference in bulk between large and small crystals is therefore much greater than a comparison of face views would suggest, and certainly the small crystals, though locally so abundant, contain only a tiny fraction of the total amount of oxalate present in the ovary.

The larger crystals of *Silybum* are amongst the biggest discovered in the course of this investigation. In particular they are much wider than is common even in crystals of similar length. Associated with this great width is an unusual variability in the form of the face. A very high proportion of crystal ends have unequal 'shoulders', so that the end point does not lie upon the mid-line of the face. With only rare and doubtful exceptions, however, the two ends of a crystal are unsymmetrical, as nearly as can be ascertained, to the same extent. A line joining the two end points will normally run parallel to the sides, though not midway between them.

Curved outlines are frequent. On the larger crystals any very pronounced curvature is rare; slight departures from rectilinearity involving not more than a quarter of the perimeter of any one crystal face are, on the other hand, quite common. The commonest type of curvature occurs at the end points, where the two 'shoulders' instead of meeting in a geometrical angle, are often drawn out into an apiculus, a little rounded prolongation of the crystalline material. One must here take into account the possibility of refraction effects giving rise to an optical illusion. Two pieces of evidence, however, have satisfied me that apiculate ends really occur. Firstly, apiculi are seen on small crystals which must be too thin to produce any appreciable optical disturbance. Secondly, a large crystal may present similar apiculate appearances at both ends, though the consideration of side views shows that optical conditions at the two ends must be quite different. In small crystals curvature is more pronounced, more extensive, and much more frequent; some of the smallest specimens have no distinct angles at all.

Amongst the larger crystals a few are found in which the sides, though quite straight, are not parallel to each other (Plate 1, Fig. 14). The angle of taper is always very small, generally much smaller than that figured, but there is no doubt of the reality of the phenomenon, which raises serious difficulties of interpretation. If we suppose these surfaces to be determined by the structure of a crystal lattice in the usual way, then we must postulate two lattices with a small angular discrepancy; if this is correct, then somewhere across the face, homogeneous though it may appear, there must exist a considerable amount of discontinuity and disorder.

Side views of the larger crystals invariably show that the faces are flat and parallel to each other. They are not, however, superposed, and the side view is never a rectangle. A simple crystal would present in side view the appearance of a parallelogram, but it is not certain that simple crystals occur in this species. Almost always the side view shows a distinct joint plane parallel to the faces and half-way between them. The larger crystals, at any rate, are



FIGS. 1–4: crystals of *Silybum marianum*. 1. Side view of fish-tail pair. 2. Side view of concordant pair. 3. Longitudinal section of a fish-tail pair, the shaded parts being those least soluble in hydrochloric acid. H and T denote head and tail ends of the components. 4. Differential solubilities in a longitudinal section of a concordant pair. FIGS. 5–12: crystals of *Onopordon acanthium*. 5–8. Different forms in face view. 9. Single crystal in side view. 10. Side view of fish-tail pair. 11. Presumed end view. 12. Longitudinal section of fish-tail pair eroded by hydrochloric acid. Original outline not shown. Funnel-shaped craters enlarged at the joint plane.

mostly parallel pairs, and it is very likely that they all are. Crystals near the minimum size are too thin to allow of any observation on this point.

Given a parallel pair of equal parallelograms, two possibilities arise. One (Text-fig. 1) may be called a *fish-tail* pair, the other (Text-fig. 2) a *concordant* pair. In *Silybum* both types occur abundantly. My impression is that the fish-tail form is the commoner of the two, but I consider that it would be rather difficult to obtain reliable estimates of the relative frequencies. Unless conditions can be achieved in which every crystal can be classified with certainty, the results will be biased, because the fish-tail type is much easier to recognize.

A very careful search has been made for additional visible differentiation in these crystals, whether of the nature of surface markings or internal structure. Nothing has been found. The faces are quite featureless, and the side view shows nothing more than the joint plane between the two components of the pair. The interior seems to be optically uniform.

When the crystals are heated their surfaces become finely striated, the striations being apparently cracks. On the faces the striations run transversely; on the sides they run parallel to the oblique ends of the crystal components. More severe heating produces conspicuous fractures which run predominantly, though imperfectly, in the directions defined by the system of striations. The study of heated specimens therefore reveals little that is new, though the application of heat may be useful in making the distinction between concordant and fish-tail pairs more conspicuous (Plate 2, Figs. 15, 16, and 17).

The application of hydrochloric acid reveals in the larger crystals an elaborate and remarkably constant structural organization. The first attack of the acid is sharply concentrated upon two elliptical areas, one upon each face of the crystal. Each of these areas is eaten out into a large cavity, which will be called a *crater*. A crater seems always to lie upon the midline of the face; even when the ends of the crystal are very unsymmetrical it is impossible to detect with certainty any lateral displacement of the crater. But a crater is not always, or even usually, equidistant from the two ends of the crystal (Plate 2, Fig. 18). It seems to be a universal rule, applying to concordant and fish-tail pairs alike, that the craters in the two components shall be displaced towards the same end of the pair. In a concordant pair this means displacement towards non-homologous ends of the two components.

Although the details are obscure, there seems to be little doubt that a crater is formed in the first place by the appearance of a number of smaller holes drilled into the face in an oblique direction, and then enlarged until they meet. The formation of a crater is, however, a very rapid process, and the material which is removed must be many times more soluble than that which makes up the main body of the crystal.

In side view a crater is unsymmetrical (Text-figs. 3 and 4). Let us, in a fish-tail pair, designate the end with the shallow sinus as the *tail* of the crystal, the end with the blunt projecting wedge as the *head*. These terms can then be transferred to the appropriate ends of the individual components, and thence to the components of a concordant pair, as indicated by the letters in the text-figures. It is a constant rule that the wall of the crater which is directed towards the head of the component is steep (indeed it is undercut), while the wall which is towards the tail of the component slopes more gently. The shape of the crater is substantially the same in fish-tail and concordant pairs, and it seems to be independent of the position of the crater (that is to say, whether the crater is nearer to the head or to the tail of the component).

Phenomena of differential solubility are not confined to the region of the crater. At the head of each component hydrochloric acid attacks the face very rapidly. This solubility is quite local, falling off swiftly and smoothly towards the tail. In the region of the crater, and from thence backwards to the tail, the main area of the face must be reckoned highly resistant to the acid. The total effect of all the differences in solubility is shown in Text-figs. 3 and 4, where the original form of the crystal is shown in white, the more resistant material being shaded.

In fish-tail pairs the action of the acid is always beautifully regular, the side view being rapidly converted into a characteristic rocket-shaped outline which then persists for some time (Plate 2, Fig. 19). In concordant pairs the two craters do not meet accurately and internal shelves or ledges are left at the joint plane (Plate 2, Fig. 20). The internal form of the crater is necessarily observed through an overlying mass of crystalline material in which the joint plane continues as a visible line. It is therefore very difficult to exclude the possibility that the two craters may be separated by a thin persistent diaphragm. This is a matter on which there is as yet no evidence either way, but if there is a diaphragm it must be extremely delicate.

I have entirely failed to demonstrate the existence of an envelope on the crystals of *Silybum*; this may mean only that any covering which is present is destroyed by hydrochloric acid.

Onopordon acanthium

The crystals are of fairly uniform size as seen in face view, with a maximum of about $17 \times 12\mu$. The thickness is more variable; the maximum is about 7μ, and most crystals seem to come fairly near to that thickness, but a small proportion of specimens appear to be quite thin plates.

The face is basically a lozenge, varying to an octagon by truncation of the corners (Text-figs. 5–8). Every face has a central marking which will be called the *hilum*, appearing as a short longitudinal line. The hilum is quite different in its optical properties from the central body of *Centaurea scabiosa*. It seems to be essentially a surface feature, and it is never visible in crystals seen in side view. An examination of a large number of crystal faces reveals a simple law governing the shape of the face. Truncation of the corners occurs preferentially upon the sides. Ends become truncated only where the sides are already truncate, and then usually to a smaller extent; the operation of this principle can be seen in the figures.

In side view these crystals present confusing appearances and my interpretation must not be regarded as final. Normally the faces appear to be domed rather than flat. I strongly suspect that this is an optical illusion arising by refraction, and in my diagrams (Text-figs. 9–11) the curvature is accordingly represented by a broken line. The 'side views' observed upon a slide seem to fall (if we pass over a certain proportion of ambiguous specimens) into three categories. The first (Text-fig. 9) has upon its side surfaces fine striations, which, as indicated, run obliquely and without interruption from face to face. The presumption must be that the side-corners run parallel with these striations. The ends are clearly thin knife-edges, but instead of being straight they are bevelled in the manner shown. I regard this figure as a single crystal. It is possible that it is a concordant pair, but the existence of a joint plane has not been demonstrated. Text-fig. 10 shows another type in which the surface striations run in chevrons and a joint plane is clearly visible. This, no doubt, is a fish-tail pair. The third type of 'side view' is shown in Text-fig. 11, and is, if we overlook the apparent curvature of the faces, rectangular. I incline to the opinion that most of these are not side views at all, but end views. The shape of the face makes it probable that end views will often be observed; if this is not an end view I do not know what is.

The action of hydrochloric acid upon these crystals is difficult to observe and almost impossible to photograph. Without a previous knowledge of *Silybum* I should not have understood it at all. As in *Silybum* the acid attacks differentially a restricted area of the face. In face view the hilum can be seen to open out rapidly into a large elliptical hole, the crystal then appearing as a ring. The erosion upon the outer edge of the face is less regular, but sometimes one can see evidence, similar to that already described in *Centaurea scabiosa*, for the existence of a system of strata or laminations. When these appear, they are always longitudinal, that is, parallel to the hilum.

Seen in side view, crystals eroded by hydrochloric acid show a crater structure not fundamentally different from that of *Silybum*. Each crater is an oblique funnel, and in a fish-tail pair the two craters converge as one would expect. What happens in single crystals, or in concordant pairs, if such exist, is still uncertain. At least in fish-tail pairs the faces themselves display a solubility gradient similar to that which occurs at the head of each component in *Silybum*. In *Onopordon*, however, this gradient extends for the full length of each component, so that a fish-tail pair, instead of being tapered by the acid only at the head, is tapered all along its length, becoming completely wedge-shaped (Text-fig. 12). Again, the position in other crystals remains obscure. The technical difficulties which arise in this work are not entirely due to the small size of the specimens. The highly soluble material forms a much greater fraction of the total bulk than it does in the two species previously described. In particular the joint plane of a pair seems to be specially vulnerable, the acid penetrating it rapidly both from the outside and from the bottoms of the craters. It is therefore common for a specimen to disintegrate before its structure can be understood. The highly symmetrical configuration of a fish-tail pair is instantly recognized, but anything else is difficult to catch at the critical moment.

Apparently the crystal of *Onopordon* has an envelope, thinner and more difficult to observe than that of *Centaurea scabiosa*, from which it differs also in its ability to withstand, at least for a time, the action of concentrated sulphuric acid.

Carthamus tinctorius

The crystals of this species are extraordinarily variable in all respects, and a complete analysis of the crystal population of the ovary would be a major undertaking. For our present purpose it will suffice to describe three major crystal types which effectively determine the general character of the population. They are accompanied by a bewildering array of intermediates and aberrant forms.

We have firstly a rather small cluster or druse crystal of very familiar pattern which seems to be quite similar to those of many other unrelated angiosperms (Plate 2, Fig. 21). It is of interest here mainly because cluster crystals are not otherwise known to occur in the ovaries of Cynareae.

The second type of crystal in many respects resembles that of *Centaurea scabiosa*. The face, though much less regular in form than that of *Centaurea*, is essentially curvilinear in outline, and there is an immersed central body (Plate 2, Fig. 22). In side view, however, there are significant differences from *Centaurea*, for the faces are not superposed. Some specimens present the appearance of parallelograms, others display the bevelled ends which have been figured in *Onopordon* (Text-fig. 9).

The third type of crystal is a complete novelty (Plate 2, Fig. 23). It is an elongated plate, with a joint plane running across it, sometimes about the middle, sometimes nearer to one end. The ends are broadly truncate and roughly parallel to the joint. Usually the plate is widest at the joint, tapering rather irregularly towards each end. The ends are straight, but the side lines are often far from rectilinear. In its typical form this plate crystal is extremely thin and fragile. It is of course perfectly transparent and its faces appear to be flat; in liquid media it is often quite difficult to see. The examples chosen for photography were abnormally thick. They are not, however, untypical in other respects.

When plate crystals are heated they crack obliquely and can then be sorted into concordant and fish-tail pairs according to the way in which the cracks meet at the joint (Plate 2, Fig. 24). In other words, in order to establish a proper homology, the face of a *Silybum* crystal must be equated with the *end* of one of the plate crystals of *Carthamus*. I have observed (once only) a plate crystal which had three components and two joints. When this specimen was heated, the components cracked in a zigzag manner.

TAXONOMIC REVIEW

The taxonomic situation, so far as it is known, is as follows.

Of all the Cynareae which I have examined, the genus *Arctium* appears, on the grounds of floral structure, to be the most primitive form. Its ovary contains a great variety of small, roughly isodiametric crystals, among which are some almost indistinguishable from the crystals of *Onopordon acanthium*. This is the only known occurrence of this crystal type outside the genus *Onopordon* itself.

In *Onopordon*, which seems also to be a relatively primitive thistle, crystals of this same characteristic type were found in every species examined. There are, however, some interspecific differences in the crystal populations. In most species of *Onopordon* the truncation of the corners is a much less conspicuous feature than in *O. acanthium*. Furthermore, most species produce, in addition to the characteristic lozenge-shaped crystals, others of much smaller size and of a fusiform shape. Plate 2, Fig. 25, shows the two forms in *O. illyricum*.

In *Silybum*, which seems to be quite closely related to *Onopordon*, an essentially similar type of crystal structure is also found, but the shape is different. Crystals of *Silybum* are distinctly elongated, and many of them are remarkably large.

Large elongated crystals are found regularly in the more specialized thistles of the genera *Carduus* and *Cirsium*. Throughout these genera the normal type appears to be a very narrow elongated hexagon, often 40 μ long but rarely more than 5 μ broad. Such crystals have no markings of any kind and attempts to demonstrate differential solubilities have so far failed completely. *Cirsium vulgare* has crystals, still of the same type, of quite exceptional size, exceeding 90 μ in length. It is curious to note that this is the only species examined which is known to be polyploid.

These rather featureless elongated crystals occur in a very wide range of genera, and their appearance varies very little. In many cases they are deeper than they are wide, so that the side view is the one normally seen. Almost always tiny needles are present which perhaps represent early stages of development.

The type of crystal seen in *Centaurea scabiosa*, with an essentially curvilinear outline and an immersed central body, appears to be a highly specialized type. Certainly it is of very restricted distribution. In its perfect state it is known only in the one species. Related types occur in *C. calcitrapa* and *Carthamus tinctorius*. Other species of *Centaurea* seem mostly to produce elongated hexagonal crystals; the position in *Carthamus* has not been explored. It is a very striking thing that this little group of species producing the curvilinear type of crystal is characterized also by another rare condition, the presence of secretory ducts in the corolla-tube.

SUMMARY

1. The ovary wall in many Compositae contains calcium oxalate crystals, the form of which varies characteristically in different species.

2. Some of the crystals have visible markings; others reveal a complex structure when heated or treated with acids. These bodies are not simple crystals, but organized inclusions of the cell.

3. In *Onopordon* and *Silybum* hydrochloric acid has a selective effect upon specific areas of the crystal surface, producing craters of characteristic form in the body of the crystal.

4. In *Centaurea scabiosa* hydrochloric acid has a selective effect of a quite

different kind. The type of crystal structure found in this species is of very restricted distribution, and is associated in all known cases with another uncommon feature, the presence of secretory ducts in the flower.

5. *Carthamus tinctorius* produces a variety of crystal types, one of which is not yet known in any other species.

6. *Cirsium vulgare*, which is a polyploid, produces exceptionally large crystals.

EXPLANATION OF PLATES 1 AND 2

PLATE 1

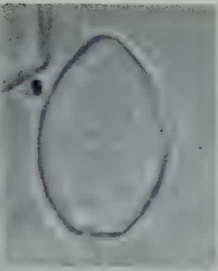
Fig. 13 × 1,000, others × 2,000.

FIGS. 1–12: crystals of *Centaurea scabiosa*. 1. Face view. 2. Side view. 3. Abnormally wide crystal. 4. Early stage in erosion by hydrochloric acid, face view. 5. Same in side view. 6. Face view showing grooves etched by hydrochloric acid. 7. Two crystals in optical section showing transverse stratification. 8. Crystal bitten in half by hydrochloric acid. 9. Two residual crystal masses within the envelope. 10. Heated crystal in side view showing joint plane. 11. Heated crystal in face view showing longitudinal striations. 12. Side view of crystal treated with sulphuric acid. FIGS. 13 and 14: crystals of *Silybum marianum*. 13. Face view. 14. Specimen with non-parallel sides.

PLATE 2

Figs. 15–20 × 1,000, others × 2,000.

FIGS. 15–20: crystals of *Silybum marianum*. 15. Face view of heated crystal. 16. Side view of heated fish-tail pair. 17. Side view of heated concordant pair. 18. Face view of crystal with crater formed by the action of hydrochloric acid. 19. Optical longitudinal section of fish-tail pair attacked by hydrochloric acid. 20. Concordant pair similarly eroded. FIGS. 21–24: crystals of *Carthamus tinctorius*. 21. Cluster crystal. 22. Curvilinear crystal with central body. 23. Plate crystal. 24. Plate crystal heated. 25. Crystals of *Onopordon illyricum*.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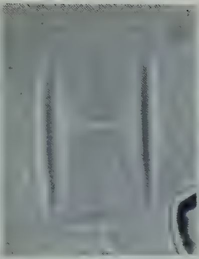
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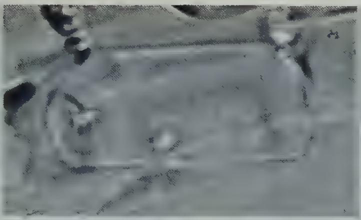
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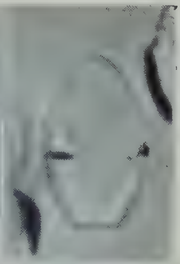
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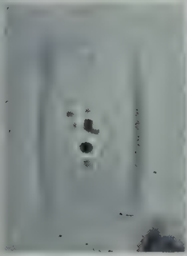
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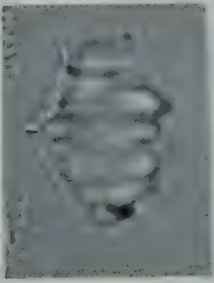
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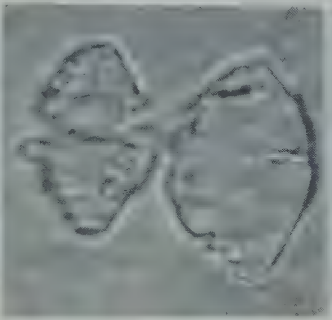
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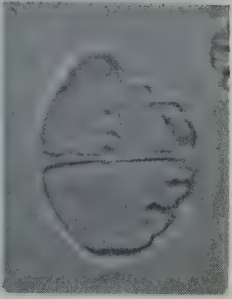
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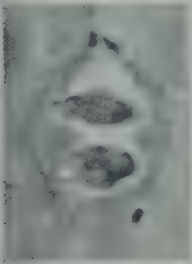
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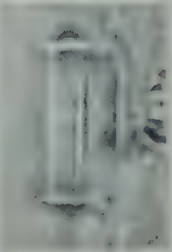
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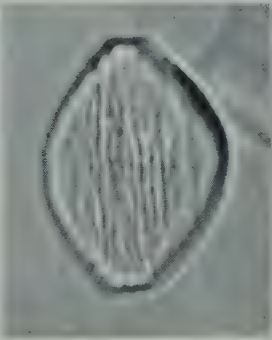
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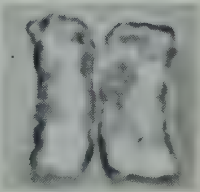
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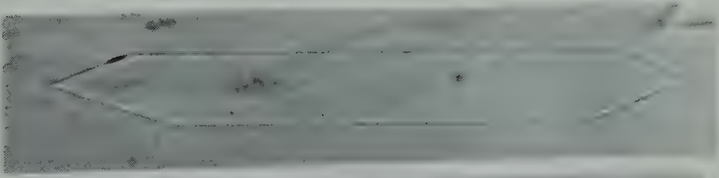
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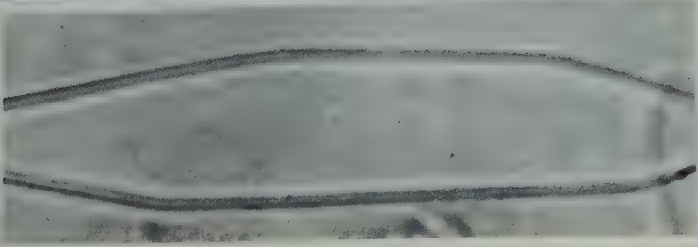
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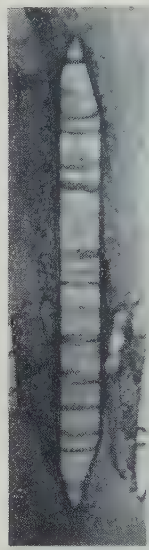
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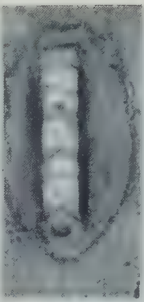
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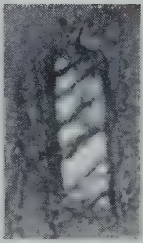
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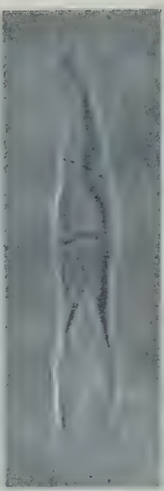
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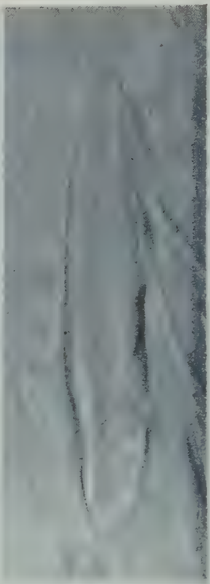
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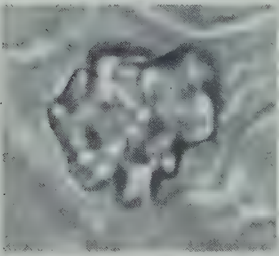
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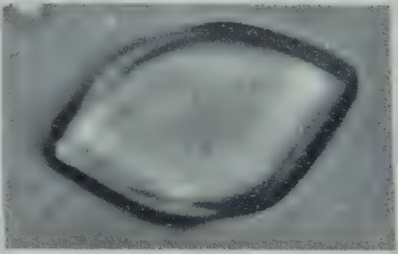
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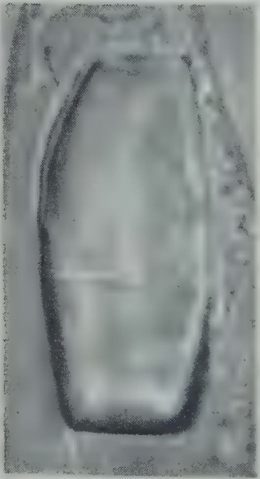
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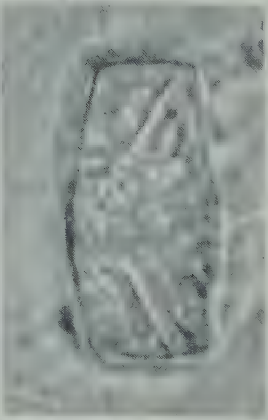
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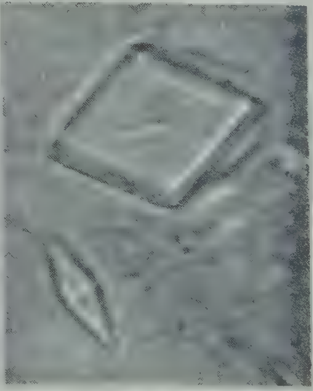
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CONTENTS

VOL. 49, No. 1 FEBRUARY 1961

T. A. and ANNE STEPHENSON. Life between tide-marks in North America. IVa. Vancouver Island, I.

F. E. ROUND. Studies on bottom-living algae in some lakes of the English Lake District. Part V. The seasonal cycles of the Cyanophyceae.

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